ROBUST SUMMARY
OF INFORMATION ON

Substance Group # Gas oils

Summary prepared by American Petroleum Institute

Creation date: February 27, 2002

Printing date: November 6, 2003

Date of last Update: **November 3, 2003**

Number of pages: **65**

NB. Reliability of data included in this summary has been assessed using the approach described by Klimisch et al.

Klimisch, H. J., Andreae, M. and Tillman, U, (1997)
A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.
Regulatory Toxicology and Pharmacology 25, 1-5.

1.1.1 GENERAL SUBSTANCE INFORMATION

Substance type : Petroleum product
Physical status : Liquid

Remark : Gas oils are complex mixtures of hydrocarbons that boil over the range 150 to 450 °C and with carbon numbers predominantly in the range C9 to C30.

Gas oils contain straight and branched chain alkanes, cycloalkanes, aromatic hydrocarbons and mixed aromatic cycloalkanes. Olefins will also be present in gas oils that have been produced from a cracking process but in general are only present in small quantities.
Some gas oils (straight run materials) contain 2- and 3-ring PACs with relatively low concentrations of 4- to 6-ring PACs. Whereas heavier atmospheric, vacuum or cracked gas oil streams will contain higher levels of the 4- to 6-ring PACs.

Mammalian and geno- toxicity studies have been conducted on a number of gas oil samples. All of the samples have been characterized in detail and this information has been published as follows:

API samples	(API 1987)
DGMK Samples	(DGMK 1991)
CONCAWE samples	(CONCAWE 91/51)
MOBIL samples	(Feuston et al, 1994)
ARCO samples	(In relevant study report)

Although the CAS numbers may not have been included in some of the above reports the samples were all described in a generic sense and related to their method of manufacture e.g. straight run or cracked gas oil. Furthermore, all the reports clearly identified the samples as gas/fuel oils, and provided sufficient information to allow the test materials to be assigned to the appropriate grouping, "predominantly saturates" or "predominantly aromatics".

Of particular importance is the information on hydrocarbon types and this information is tabulated below. The samples have been subdivided into those that contain predominantly saturates or predominantly aromatics.

Sample (CAS No.)	**Saturates**	**Aromatics**	**Olefins**
Predominantly Aromatic			
MOBIL LCO* (64741-59-9)	20.2	79.8	0
API 83-07 (64741-59-9)	24	72.4	3.7
CONCAWE MD7 (64741-59-9)	29.1	69.1	1.8
API 83-08 (64741-59-9)	31.4	60.8	7.8
DGMK 10	40.2	59.8	0
DGMK 7	44.9	55.1	0
DGMK 14	47.6	52.4	0
DGMK 6	47.9	48.3	3.8

Predominantly saturates			
DGMK 13	52.5	46.9	0.6
DGMK 12	56.1	42.6	1.3
MOBIL CLGO* (64741-82-8)			
	56.9	43.1	0
DGMK 9	57.5	42.5	0
DGMK 2	65.3	32.6	2.1
ARCO F-215	65.4	34.6	0
API 81-10 (64742-80-9)			
	65.6	34.4	0
DGMK 5	66.1	33.9	0
DGMK 4	70.7	29.3	0
DGMK 3	71.9	28.1	0
DGMK 8	73	27	0
CONCAWE MD6 (64742-46-7)			
	73.8	22.6	3.6
DGMK 11	74.6	21	4.4
DGMK 1	76.4	23.6	0
ARCO F-220	77.7	22.3	0
API 83-11 (64741-44-2)			
	78.8	21.2	0
API 81-09 (64742-80-9)			
	79.4	20.6	0
ARCO F-188	86	14	0

* LCO = Light cycle oil
CLGO = Coker light gas oil

For most of the mammalian toxicology endpoints, information has been used that was derived by the American Petroleum Institute on a wide range of gas oil streams. For simplicity, this robust summary contains detailed information on a single API sample for each endpoint and if data were available on other samples for the same endpoint they have been summarized in tabular form in the relevant sections or discussed in detail when appropriate.

(30) (43) (52)

1.13 REVIEWS

Memo : CONCAWE

Remark : CONCAWE has summarized the available health effects data and also data on environmental effects in a non-critical review.

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Memo : ATSDR

Remark : ATSDR published a toxicology review on fuel oils.

(40)

Memo : IARC

Remark : IARC reviewed the available data on distillate fuels and assessed the strength of evidence that the fuels were a carcinogenic risk to man and animals.

The conclusions of the IARC review were:

Evaluation:
There is inadequate evidence for the carcinogenicity in humans of diesel fuels.

There is limited evidence for the carcinogenicity in experimental animals of marine diesel fuel.

There is limited evidence for the carcinogenicity in experimental animals of fuel oil No. 2.

The overall evaluations were:

Marine diesel fuel is possibly carcinogenic to humans (Group 2B)

Distillate (light) diesel fuels are not classifiable as to their carcinogenicity to humans (Group 3)

Distillate (light) fuel oils are not classifiable as to their carcinogenicity to humans (Group 3)

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2.1 MELTING POINT

Method : ASTM D97
GLP : No data

Remark : Melting point is the temperature at which a solid becomes a liquid at normal atmospheric pressure. For complex mixtures like petroleum products, melting point may be characterized by a range of temperatures reflecting the melting points of the individual components. To better describe phase or flow characteristics of petroleum products, the pour point is routinely used. The pour point is the lowest temperature at which movement of the test specimen is observed under prescribed conditions of the test (ASTM 1999). The pour point methodology also measures a "no-flow" point, defined as the temperature of the test specimen at which a wax crystal structure and/or viscosity increase such that movement of the surface of the test specimen is impeded under the conditions of the test (ASTM 1999). Because not all petroleum products contain wax in their composition, the pour point determination encompasses change in physical state (i.e., crystal formation) and/or viscosity property.

The following pour point data reflect characteristic values for high aromatic gas oils. Values typical for high saturate gas oils will be similar to those of the distillate fuels, which generally fall into the high saturate subcategory. However, the pour point values of some distillate fuels may be lower than a corresponding gas oil due to the addition of additives designed to lower the fuel's pour point.

Result : Light Cat-Cracked Distillate
(CAS No 64741-59-9)

Sample	Pour point (°C)	Method	Ref
API 83-07	-12	ASTM D97	API 1987
API 83-08	-15	ASTM D97	API 1987

Reliability : (2) valid with restrictions
Results of standard method testing was reported in a reliable review dossier and reference database.

(30) (38)

2.2 BOILING POINT

Method : ASTM D86
GLP : No data

Result :

Sample	Boiling Range °C	Method	Ref.
Predominantly aromatics			
Light Cat-Cracked Distillate (CAS No 64741-59-9)			
API 83-07	240-372	ASTM D86	API 1987
API 83-08	185-351	ASTM D86	API 1987

Predominantly saturates
Hydrodesulfurized Gas Oil (CAS No. 64742-80-9)

API 81-09	261-301	ASTM D86	API 1987
API 81-10	172-344	ASTM D86	API 1987

Straight-Run Gas Oil (CAS No. 64741-44-2)

API 83-11	185-391	ASTM D86	API 1987

Reliability : (2) valid with restrictions
Results of standard method testing were reported in a reliable review dossier and a reference database.

(30)

2.4 VAPOUR PRESSURE

Value : at 25 °C
Method : Estimated (EPIWIN, MPBPWIN V1.40; U.S. EPA 2000)
GLP : N

Remark : It is predicted from vapor pressure modeling that C9 to C30 paraffinic, naphthenic, olefinic and aromatic hydrocarbon components of gas oils will have approximate vapor pressure values <2 kPa at 25 °C. As hydrocarbon chain lengths exceed C15, vapor pressures fall below levels capable of being measured by standardized techniques (OECD Guideline 104, Vapor Pressure; OECD 1995). The vapor pressure of complex mixtures is equal to the sum of the vapor pressures of the individual constituents in their pure form times their mole fraction in the mixture (Raoult's Law). Therefore, the total vapor pressure of a gas oil will depend on the proportion of different molecular weight constituents making up the mixture. These estimated vapor pressures of component hydrocarbons in gas oils are generally within the range and support the measured values for distillate fuels.

Result :

Vapor Pressure Estimates (kPa)

	Number of C Atoms		
	C9	**C15**	**C30**
Paraffins			
n-	0.6	$5x10^{-4}$	$5x10^{-8}$
iso-	1.3	$6x10^{-3}$	$2x10^{-8}$
Naphthenes			
1-ring	0.6	$3x10^{-4}$	$6x10^{-9}$
2-ring	0.3	$3x10^{-3}$	$7x10^{-9}$
Olefins			
straight	0.7	$6x10^{-4}$	$9x10^{-9}$
cyclic	0.4	$2x10^{-3}$	$6x10^{-9}$
Aromatics			
1-ring	0.5	$8x10^{-4}$	$9x10^{-10}$
2-ring	0.01 (1)	$5x10^{-5}$	$1x10^{-10}$

(1) 10 carbon atoms in structure

Reliability : (2) valid with restrictions
Estimations made using a validated computer model

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2.5 PARTITION COEFFICIENT

Partition coefficient : Octanol-water
Method : Calculated by KOWWIN, V 1.66 (EPIWIN V 3.10; EPA 2001)
Year : 2001
GLP : No

Remark : Kow values of representative C9 and C30 paraffinic, naphthenic, olefinic and aromatic hydrocarbon components of gas oils were modeled. From the carbon number range of modeled individual hydrocarbon structures, the apparent range of Kow values of these substances will extend from 3.3 to >6 (API 1987).

The modeled values given above are consistent with the Kow estimates of 3.9 to >6 calculated by CONCAWE (1996) based on known hydrocarbon composition of a hydrodesulfurized gas oil (CAS No. 64742-80-9), a straight-run gas oil (CAS No. 64741-44-2) and a light catalytic cracked gas oil (CAS No. 64741-59-9). These Kow estimates cover gas oil streams having both high saturate and high aromatic fractions.

Result :

Log Kow = 3.3 to approximately 13

	Log Kow values No. of C atoms	
	C9	**C30**
Paraffins		
n-	4.8	16
iso-	4.7	15
Naphthenes		
1-ring	4.6	15
2-ring	3.7	14
Olefins		
straight 5.2	15	
cyclic	4.5	13
Aromatics		
1-ring	3.7	14
2-ring	3.3*	13

* Value given for a C10 molecule

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model.
(30) (44) (45) (64)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water
Value : < 0 .001 mg/l at 25 °C
Method : Calculations by WSKOW V 1.40. (EPIWIN V 3.10; EPA 2001
Year : 2001
GLP : No

Remark : It is predicted from solubility modeling that C9 to C30 paraffinic,

naphthenic, olefinic and aromatic hydrocarbon components of gas oils will have approximate water solubility values of 0.1 to approximately 52 mg/l at 25 °C. As hydrocarbon chain lengths exceed C15, these compounds become increasingly hydrophobic to the point where solubility becomes negligible. Hydrocarbon components of gas oils in the range C16 to C30 typically have water solubility values less than 0.1 mg/l. The ultimate solubility of a specific gas oil will depend not only on the relative percentage of aromatic versus saturate fractions but also on the molecular weights of those principal hydrocarbons.

Result :

Water solubility estimates (mg/l)

	No. of C atoms C9	**C15**	**C30**
Paraffins			
n-	2.3	<0.001	<0.001
iso-	2.7	<0.001	<0.001
Naphthenes			
1-ring	3.4	0.004	<0.001
2-ring	19	0.26	<0.001
Olefins			
straight 1.1	0.004	<0.001	
cyclic	4.1	0.005	<0.001
Aromatics			
1-ring	52	0.035	<0.001
2-ring	31*	0.63	<0.001

* Value given for a C10 molecule

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model.
(65)

3.1.1 PHOTODEGRADATION

Type : Calculated
Method : Calculated: by AOPWIN V 1.90 (EPIWIN V 3.10; EPA 2001
Year : 2001
GLP : No
Test substance : TS: Gas oils, various

Remark : Direct photolysis is not expected to be a major degradation pathway for most of the components in gas oils. Chemicals having the potential to photolyze have UV/visible absorption maxima in the range of 290 to 800 nm. Most hydrocarbon constituents in this category are not expected to photolyze since they do not show absorbance within the 290-800 range. However, where unsaturated hydrocarbons, notably aromatic hydrocarbons are present in, or near the surface of water, degradation by reaction with sunlight in the presence of oxygen can be a significant removal process (CONCAWE 2001).

It is predicted from indirect photolysis modeling of C9 to C30 paraffinic, naphthenic, olefinic and aromatic hydrocarbon compounds that volatile components in gas oils will undergo moderate atmospheric oxidation and not persist in the environment. Base on the modeled half-life values of component hydrocarbon structures, gas oils containing primarily aromatic or saturate fractions are not expected to show significant differences in their photodegradation characteristics.

Result : Direct photolysis:
Most substances in this category are not subject to direct photolysis; see remarks section below.

Half-life	N/A
Degradation	N/A
Quantum yield	N/A

Indirect photolysis:

Sensitizer type	Hydroxyl radicals (OH-)
Conc. of sensitizer	1.5×10^6 OH^-/cm^3
Rate constant	various
Half-life	see table of half-lives below (given in days)
Breakdown products	N/A

	Half life values (days) No. of C atoms		
	C9	**C15**	**C30**
Paraffins			
n-	1.1	0.6	0.3
iso-	1.1	0.6	0.3
Naphthenes			
1-ring	0.8	0.5	0.2
2-ring	0.8	0.4	0.2
Olefins			
straight	0.3	0.2	0.2
cyclic	0.1	0.1	0.1
Aromatics			
1-ring	1.5	0.7	0.3

2-ring	0.5*	0.2	0.1

* Value given for a C10 molecule

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model.
(45) (63)

3.1.2 STABILITY IN WATER

GLP : No

Remark : Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters. The chemical components found in the materials that comprise the gas oil category are hydrocarbons that are not subject to hydrolysis because they lack functional groups that hydrolyze.

Reliability : (1) valid without restriction
(53)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : Calculated

Method : Calculations by fugacity-based environmental equilibrium partitioning model (EQC model)

Remark : Multimedia distribution was calculated for low and high molecular weight hydrocarbon compounds representing common PONA (i.e., paraffinic, olefinic, naphthenic and aromatic) constituents in gas oil streams. Partitioning behavior depends largely on molecular weight, with smaller compounds (e.g., 9 to 15 carbon atoms) partitioning to the air due to relatively high vapor pressures. Here they are expected to degrade rapidly via hydroxyl radical attack (indirect photodegradation). Larger molecular weight hydrocarbons (e.g., 15 to 30 carbon atoms) partition to the terrestrial environment where they are expected to undergo slow biodegradation. Mobility in the aquatic environment is low due to low water solubility or to high vapor pressure in those compounds showing appreciable water solubility limits. Based on the relative distribution of component hydrocarbons, gas oils having principally aromatic or saturate fractions are not expected to show significant differences in environmental partitioning.

Result : Media:
Air, Water, Soil, Sediment, Suspended Sediment, Fish.

PERCENT DISTRIBUTION

	Air	Water	Soil	Sed.	Susp. Sed.	Fish
n-Paraffins						
C9	99	<0.1	1	<0.1	<0.1	<0.1
C15	13	<0.1	85	2	<0.1	<0.1
C30	<0.1	<0.1	98	2	<0.1	<0.1

Iso-paraffins						
C9	99	<0.1	0.5	<0.1	<0.1	<0.1
C15	68	<0.1	31	0.7	<0.1	<0.1
C30	0.1	<0.1	98	2	<0.1	<0.1
Straight olefins						
C9	99	<0.1	0.7	<0.1	<0.1	<0.1
C15	17	<0.1	81	2	<0.1	<0.1
C30	<0.1	<0.1	98	2	<0.1	<0.1
Cyclic olefins						
C9	99	0.3	0.7	<0.1	<0.1	<0.1
C15	49	<0.1	50	1	<0.1	<0.1
C30	<0.1	<0.1	98	2	<0.1	<0.1
1-ring naphthenes						
C9	99	<0.1	0.9	<0.1	<0.1	<0.1
C15	0.4	<0.1	97	2	<0.1	<0.1
C30	0.1	<0.1	98	2	<0.1	<0.1
2-ring naphthenes						
C9	99	0.2	1	<0.1	<0.1	<0.1
C15	51	<0.1	48	1	<0.1	<0.1
C30	0.1	<0.1	98	2	<0.1	<0.1
1-ring aromatics						
C9	97	1	2	<0.1	<0.1	<0.1
C15	19	<0.1	79	2	<0.1	<0.1
C30	<0.1	<0.1	98	2	<0.1	<0.1
2-ring aromatics						
C10	77	8	15	0.3	<0.1	<0.1
C15	0.7	0.2	97	2	<0.1	<0.1
C30	<0.1	<0.1	98	2	<0.1	<0.1

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3.5 BIODEGRADATION

Type : Aerobic
Inoculum : Adapted inoculum of domestic activated sludge
Contact time : 28 day(s)
Method : CONCAWE. Test method for determining the inherent aerobic biodegradability of oil products. 1996/1997, and modification of ISO/DIS 14593
Year : 1993
GLP : No
Test substance : Gas oils (petroleum), solvent-refined; CAS No. 64741-90-8.

Method : Water quality-Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium-Method by analysis of inorganic carbon in sealed vessels (CO_2 headspace test)
Result : Test material was inherently biodegradable since it achieved >20% biodegradability based on CO_2 production.

	% Degradation (sd)	
Test Day	**Aniline**	**Gas oil**
7	82.98 (1.018)	35.85 (0.636)
14	78.19 (0.575)	41.96 (2.517)
21		39.40 (0.926)
28		34.82 (0.156)*

*Lower values due to recalibration using standard concentration exceeding linear range of quantitation.

Source : American Petroleum Institute.

Test condition : Acclimated inoculum prepared from activated sludge was incubated with test substance and aniline (positive control) during a two week adaptation period in separate test systems. Test medium consisted of 1 liter glass distilled water and mineral salts (10 ml/l phosphate buffer, 1ml 0.025% ferric chloride, 1ml 2.75 magnesium sulfate, and 1 ml 2.75% calcium chloride solutions) prepared as described in ISO method.

Acclimation of inoculum was performed using activated sludge from the aeration basin of Somerset-Raritan Valley Wastewater Treatment Plant (Bridgeport, NJ., U.S.A.). On the day of collection, the sludge was returned to the laboratory and homogenized for two minutes in a blender at medium speed. The supernatant was removed from the homogenated sample after a settling period of at least 30 minutes and then filtered through Whatman #4 coarse filter paper. One hundred mls of the filtered sludge was combined with 900 ml test medium in a 2 liter flask for each of the two acclimation test systems. Gas oil or aniline were administered to the appropriate flask on days 0, 7 and 11 to achieve approximately 4, 8 and 8 mg of carbon as test or positive control substance. The flasks walls were covered to prevent light exposure to the inoculum, loosely stoppered with gauze to allow aeration and placed on a gyratory shaker set at 200 rpm. An aqueous stock solution of aniline was used to dose the positive control acclimating inoculum. The gas oil was introduced as neat material using a tared 10 µl Hamilton glass syringe, and amounts added were determined on a gravimetric basis.

On day 14 of incubation gas oil acclimated inoculum was transferred to a separatory funnel and approximately half the volume of aqueous phase was drained from the funnel for testing, avoiding any carryover of residual gas oil. The drained gas oil inoculum was combined with an equal volume of the aniline acclimated inoculum and mixed thoroughly. One hundred ml of the composite inoculum was mixed with 900 ml test medium, and then used to prepare the biodegradation test systems.

Biodegradation test vessels (125 ml serum bottles) were filled with 100 ml of the inoculated test medium. Blank test systems received no additional treatment and were sealed immediately. Addition of the respective substances was performed as described for the acclimation procedure to achieve test concentrations of approximately 10 mg carbon/l. Duplicate test systems for gas oil, aniline and blank treatments were prepared for sacrifice at weekly sampling intervals for subsequent CO_2 analysis. All samples not designated for time zero analysis were crimp sealed, covered with aluminum foil and agitated on a gyratory shaker at approximately 200 rpm. CO_2 determination was performed on days 0, 7, 14, 21 and 28. At each sampling interval, the appropriate test systems were basified with 0.5 ml of 10N NaOH (pH>12) in order to convert CO_2 in the headspace to water soluble sodium carbonate, returned to the gyratory shaker for an

hour and then analyzed. Total inorganic carbon (TIC) in the aqueous phase was measured using an O.I. Model 700 carbon analyzer calibrated with sodium carbonate standards. On days 0, 7, 14 and 21 standard concentrations used were suitable to quantify up to 25 ppm carbon, on day 28 calibration curve was developed to quantify up to 50 ppm carbon.

Reliability : (2) valid with restrictions
The data reported in this report were obtained through participation with CONCAWE task group for inherent biodegradation method development of water insoluble substances. The gas oil sample was supplied by Burmah Castrol as part of research project to identify procedures for inherent biodegradation test Thus, although the work was conducted according to relevant test guidelines and standard operating procedures, the research was not done in strict accordance with GLPs.

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5.1.1 ACUTE ORAL TOXICITY

Type : LD_{50}
Value : 6790 - 7180 mg/kg bw
Species : Rat
Strain : Sprague-Dawley
Sex : Male/female
Number of animals : 5
Vehicle : None
Year : 1985
GLP : Yes
Test substance : API 83-08, predominantly aromatic, see section 1.1.1.

Method : The test material was administered undiluted, as a single oral dose to groups of 5 male at each of 5 dose levels and 5 female rats at each of 3 dose levels. The rats had been fasted for 24 hours prior to dosing but had free access to water.
Following dosing, food and water were available ad-lib for a period of 14 days. The animals were observed for clinical signs of toxicity and mortality every hour for the first 6 hours after dosing and twice daily thereafter for 14 days. The rats were weighed just prior to dosing and then at 7 and 14 days after dosing.
At study termination all animals were killed with carbon dioxide and subjected to a gross necropsy and abnormalities were recorded.
The LD_{50} and 95% confidence limits were calculated using a standard technique.

Result : Clinical signs seen during the study included: hypoactivity, diarrhea, yellow-stained urogenital/abdominal area, hair loss on anal region/abdomen/hind legs, ataxia, red-stained nose and mouth, prostration, lacrimation, catalepsy, dyspnea, possible respiratory congestion, hypothermic to touch, inflamed anal region and death.
Mortalities were as follows:

Dose (g/kg)	No. dead/ No. dosed
Males	
5.0	0/5
6.25	3/5
7.81	3/5
9.76	3/5
12.2	5/5
Females	
5.0	1/5
6.25	1/5
7.81	4/5

Reliability : (1) valid without restriction

(18)

Type : LD_{50}
Species : Rat
Test substance : Gas oils various. See section 1.1.1

Result : In addition to the study summarized above, the American Petroleum Institute reported acute oral toxicity studies for four other gas oils. In all five studies the clinical signs were similar irrespective of the test material.

All studies are considered to be reliability 1 (Klimisch) and therefore only one study has been described fully in the robust summary above.

The following table summarizes the LD_{50} data available for all five studies (including API 83-08) that have been reported.

Sample	CAS No.	Oral LD_{50} (g/kg)	Reference
Predominantly aromatic			
API 83-07	64741-59-9	M 4.66 F 3.2	API 33-30162
API 83-08	64741-59-9	M 7.18 F 6.79	API 32-32859
Predominantly saturates			
API 81-10	64742-80-9	>5.0	API 30-32348
API 83-11	64742-44-2	>5.0	API 32-32857
API 81-09	64742-80-9	>5.0	API 30-32347

Reliability : (1) valid without restriction

(2) (4) (5) (19)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC_{50}
Species : Rat
Strain : Sprague-Dawley
Sex : Male/female
Number of animals : 5
Exposure time : 4 hour(s)
Year : 1987
GLP : Yes
Test substance : API 83-11, predominantly saturates, see section 1.1.1.

Method : 5 male and 5 female rats were exposed to 6 different concentrations of test substance by whole body exposure for 4 hours. The study was conducted in two parts. The first part consisted of exposures at a single nominal concentration of 5 mg/l, the second part consisted of exposures at nominal concentrations of 0, 3.3, 4.78, 6.55 and 7.58 mg/l. After exposure for 4 hours, the rats were observed twice daily for mortality. The animals were weighed prior to exposure and again on days 7 and 14 post exposure. On day 14 all surviving animals were killed by exsanguination following methoxyflurane anesthesia. For all animals, including those found dead during the study the lungs and any grossly abnormal tissues were removed, fixed and examined histologically.

Result : Results of chamber monitoring and overall mortalities are shown in the following table

Chamber concentration (mg/l)		No. Dead/No. exposed	
Nominal	Actual	Male	Female
14.1	5.39	5/5	5/5
0.0	0.01	0/5	0/5
3.31	1.05	0/5	0/5
6.55	3.22	4/5	5/5
4.78	1.6	3/5	1/5
7.58	2.25	4/5	5/5

The actual concentrations were used to determine the LC_{50} values. These were as follows:

Sex	LC_{50} (mg/l)	95% Confidence limits (mg/l)
Male	1.72	1.22 to 2.42
Female	1.82	1.45 to 2.28
Male & Female	1.78	1.44 to 2.2

There was an apparent dose-related weight suppression at 7 days but at 14 days body weight gains were observed in surviving animals. Clinical signs during the exposure consisted of: decreased activity, wet inguinal area, eyes partially closed, wet coat and oily coat. In the seven days following exposure there were signs of poor condition and respiratory distress. In the second week survivors were considered to be normal in appearance.
Gross post mortem findings included oily hair coat which was attributable to deposition of test material. Additionally, dark red lungs were observed in all animals that died within a day or two of exposure. This was not observed in any animal that survived the exposure.
A diffuse moderate or marked pulmonary congestion and perivascular edema was seen in all animals that died during the study. Spotty alveolar edema was also seen but less consistently.
In all animals that had died during the study widespread damage had occurred to the alveolar walls resulting in fibronecrotic debris and extravasation of RBCs and PMNs. Necrosis was seen in the walls of small blood vessels and there was spotty epithelial necrosis in small bronchioles, but the most severe damage seemed to be centroacinar. Larger airways were relatively unaffected. Animals surviving to term did not exhibit the above changes but survivors that had been exposed to levels of 1.5 mg/l and above exhibited chronic inflammatory changes in the lungs.

Test condition : Exposures were conducted in stainless steel and glass chambers of 0.25 cubic meter volume. The rats were individually housed during exposure. Aerosols of API 83-11 were generated using a nebulizer and were introduced to the exposure chamber intake where it was diluted with room air to achieve the target concentration. Actual chamber concentrations were determined gravimetrically by collection of aerosols on filters.

Reliability : (1) valid without restriction

(1)

Type : LC_{50}
Test substance : Gas oils various, see section 1.1.1.

Result : The American Petroleum Institute have reported four other acute inhalation toxicity studies on gas oil samples.

The LC_{50} determined in all studies (including the one summarized above) are as follows:

Sample	CAS	LC_{50} (mg/l)	Reference
Predominantly aromatic			
API 83-07	64741-59-9	5.4*	API 33-30549
API 83-08	64741-59-9	4.65**	API 33-30444

Predominantly saturates			
API 81-10	64742-80-9	7.64	API 30-32857
API 83-11	64742-44-2	M 1.72 F 1.82 M&F 1.78	API 34-3065
API 81-09	64742-80-9	4.60	API 30-32856

* LC_{50} 5.4 mg/l for combined sexes.
3.35 mg/l for males; female data inadequate to calculate female LC_{50} alone.

** No deaths occurred during the exposure period at 5.06 mg/l aerosol. However, 3 males and 1 female from this group died during the 14-day observation period. (Repeated at 2.34 - 7.29 mg/l) LC_{50} of 4.65 mg/l for combined sexes but data insufficient for determination of LC_{50} for females alone.

Reliability : (1) valid without restriction

(8) (9) (25) (26)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD_{50}
Value : > 2000 mg/kg bw
Species : Rabbit
Strain : New Zealand white
Sex : Male/female
Number of animals : 8
Year : 1985
GLP : Yes
Test substance : TS: API 83-08, predominantly aromatic, see section 1.1.1.

Method : A weighed quantity of undiluted test material (equivalent to a dose of 2 g/kg) was applied to the dorsal skin of each of 4 male and 4 female rabbits. The skin of the patched area of two rabbits of each sex had been abraded whilst the other two had intact skin. The applied dose was covered with an occlusive dressing (gauze and an impermeable covering). 24 hours after dosing, the patches were removed, the skin wiped and collars fitted to the rabbits to prevent oral intake of any residual test material. The collars were removed 24 hours later and the animals were observed for a total of 14 days post-dosing.
At study termination the animals were killed with carbon dioxide and a gross necropsy was performed. Any abnormalities were recorded.

Result : No signs of systemic toxicity or death occurred at the dose level of 2 g/kg in this study.
Dermal irritation ranged from slight to severe for erythema and edema, from slight to moderate for atonia, desquamation and coriaceousness, and from slight to marked for fissuring. Other dermal irritation seen included subcutaneous hemorrhage and blanching.

Conclusion : The LD_{50} was greater than 2 g/Kg in both sexes for both intact and abraded skin.

Reliability : (1) valid without restriction

(18)

Type : LD_{50}
Test substance : Gas oils various, see section 1.1.1.

Result : The American Petroleum Institute reported five acute dermal toxicity studies for gas oils including sample API 83-08 (described above).
In all studies the clinical signs were similar irrespective of the test material.
All studies are considered to be reliability 1 (Klimisch) and therefore only one study has been described fully in the robust summary above.

The following table summarizes the LD_{50} data available for all five studies (including API 83-08) that have been reported.

Sample	CAS No.	Dermal LD_{50} (g/kg)	Reference
Predominantly aromatic			
API 83-07	64741-59-9	>2.0	API 33-30162
API 83-08	64741-59-9	>2.0	API 32-32859
Predominantly saturates			
API 81-10	64742-80-9	>2.0	API 30-32348
API 83-11	64742-44-2	>2.0	API 32-32857
API 81-09	64742-80-9	>2.0	API 30-32347

Reliability : (1) valid without restriction

(2) (4) (5) (19)

5.2.1 SKIN IRRITATION

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
PDII : 6.9
Method : Draize Test
Year : 1985
GLP : Yes
Test substance : API 83-08, predominantly aromatic, see section 1.1.1.

Method : 0.5 ml of undiluted test material was applied to two areas on each of six rabbits. One area was intact and the other abraded skin. The treated area was then covered with an occlusive dressing.
After 24 hours the dressing was removed and the treated skin was wiped to remove any residue of test material. The degree of erythema and edema was recorded according to the Draize scale. A second reading of skin responses was made at 72 hours again at 96 hours, 7 and 14 days.
Results of the 24 and 72 hour readings were used to determine the Primary Irritation Index.

Result : Moderate to severe levels of irritation occurred in this study.
There was no real difference in the severity of the response on either intact or abraded skin. Average irritation scores were as follows for abraded skin:

	Erythema	Edema
24 hours	3.2	3.2
72 hours	3.7	3.7
96 hours	3.5	3.3
7 days	2.2	1.7
14 days	0.3	0.0

Blanching was seen in 2 animals at 24 hours and in six animals at 72 hours. At 96 hours subcutaneous hemorrhaging within the test sites was

observed in all animals A possible necrotic area was seen in one animal at 96 hours.

Reliability : (1) valid without restriction

(18)

Species : Rabbit
Test substance : Gas oils various, see section 1.1.1.

Result : Seven skin irritation studies have been reported for gas oil samples, including sample API 83-08. All the available data (including that for API 83-08) are summarized below.

It is interesting to note that the predominantly aromatic gas oils were slightly more irritant than the predominantly saturate gas oils.

Sample	CAS No.	Irritation index	Reference
Predominantly aromatic			
API 83-07	64741-59-9	5.6	API 33-30162
CONCAWE MD 7	64741-59-9	*	CONCAWE 91/51
API 83-08	64741-59-9	6.9	API 32-32859
Predominantly saturates			
API 81-10	64742-80-9	5.9	API 30-32348
CONCAWE MD 6	64742-46-7	**	CONCAWE 91/51
API 83-11	64742-44-2	3.2	API 32-32857
API 81-09	64742-80-9	4.3	API 30-32347

Samples MD 6 and MD 7 were tested in a 4 hour semi-occlusive patch test in rabbits.

* MD-7 elicited well defined erythema in one animal and moderate/severe erythema in two animals at the 60 minute, Day 1, Day 2, and Day 3 observations. One animal had well defined erythema on Day 7. Edema, which ranged from very slight to moderate, was observed in all animals up till the 72 hour stage. Desquamation was seen in all animals at day 7 and in only one animal at day 10. At day 10 all animals were free from erythema and/or edema.

** MD-6 elicited minimal, transient dermal irritation in all 3 animals. At the 60 minute observation time, well-defined erythema and very slight edema was observed. Very slight erythema was observed in all animals at day 1, 2 animals on days 2 and 3, and one animal on day 7. Two animals were observed with desquamation on day 7. All animals were free of dermal abnormalities at the 10 day observation.

Reliability : (1) valid without restriction

(2) (4) (5) (19) (48) (50)

5.2.2 EYE IRRITATION

Species : Rabbit
Concentration : Undiluted
Dose : 0.1 ml
Number of animals : 9

Method : Draize Test
Year : 1985
GLP : Yes
Test substance : API 83-08, predominantly aromatic, see section 1.1.1.

Method : 0.1 ml of undiluted test material was applied to the corneal surface of one eye of each of 9 rabbits, the other eye was untreated and served as control. After 30 seconds the treated eyes of 3 rabbits were washed with lukewarm water for 1 minute. Eyes of the other 6 rabbits were not washed. Readings of ocular lesions for all animals were made at 1, 24, 48, 72 hours and 7 days after treatment. Sodium fluorescein was used to aid in revealing possible corneal injury.

Result : There was no pain response from any animal following instillation of the test material onto the cornea. No corneal irritation was seen during the study. However, a white fibrin filament was seen in the anterior chamber in one animal (unwashed eye group) at 48 and 72 hours.

Primary eye irritation scores recorded in this study are as follows:

	1 Hr.	24 Hrs	48 Hrs.	72 Hrs.	7 days
Unwashed eyes	4.3	3.2	2.2	1.2	0.0
Washed eyes	4.7	0.0	0.0	0.0	0.0

Reliability : (1) valid without restriction

(18)

Species : Rabbit
Test substance : Gas oils various, see section 1.1.1.

Result : Five eye irritation studies have been reported for gas oil samples, including sample API 83-08. The irritation scores at 24 hours (including those for API 83-08) are tabulated below.
The predominantly aromatic materials appear to be slightly more irritating than the predominantly saturate gas oils.

Sample	**CAS No.**	**Eye Irritation Index**		**Reference**
		rinsed eye	**Unrinsed eye**	
Predominantly aromatic				
API 83-07	64741-59-9	2.0	1.7	API 33-30162
API 83-08	64741-59-9	0.0	3.2	API 32-32859
Predominantly saturates				
API 81-10**	64742-80-9	0	1.0	API 30-32348
API 83-11	64742-44-2	0.0	1.0	API 32-32857
API 81-09*	64742-80-9	0	2.0	API 30-32347

* 1 hour after instillation of test material, the average eye irritation scores were 2.7 and 0.7 for the unrinsed and rinsed eyes respectively

** 1 hour after instillation of the test material, the average irritation index was 2.7 for both the rinsed and unrinsed eyes

*** average eye irritation index for 48 hour readings were 2.0 and 0.67 for unrinsed and rinsed eyes respectively

**** At 48 hours the average eye irritation index was 0 for both rinsed and unrinsed eyes

***** At 48 hours the average eye irritation index was 0 and 0.33 for rinsed and unrinsed eyes respectively

Reliability : (1) valid without restriction

(2) (4) (5) (19)

5.3 SENSITIZATION

Type : Buehler Test
Species : Guinea pig
Concentration : 1st: Induction undiluted occlusive epicutaneous
2nd: Challenge 10 % occlusive epicutaneous
Number of animals : 76
Result : not sensitizing
Year : 1985
GLP : Yes
Test substance : API 83-08, predominantly aromatic, see section 1.1.1.

Method : 0.4 ml undiluted test material was applied under an occlusive dressing to the shaved skin of 10 male and 10 female animals. Six hours after application the dressing was removed and the skin wiped to remove residues of test material. The animals received one application each week for 3 weeks. The same application site was used each time. Two weeks following the third application a challenge dose (0.4 ml of a 10% solution in paraffin oil) was applied in the same manner as the sensitizing doses. A previously untreated site was used for the challenge application.
The application sites for sensitizing and challenge doses were read for erythema and edema 24 and 48 hours after patch removal. To assist in the reading of the response to the final challenge dose the test site was depilated 3 hours prior to reading by using a commercially available depilatory cream.

Positive control, vehicle control and naive control groups were included in this study.

Concentrations of positive control were as follows:

Sensitizing doses: 0.4 ml of 0.3% w/v in 80% aqueous ethanol
Challenge dose: 0.4 ml of 0.1% w/v suspension in acetone

Result : A mixture of clinical signs were observed during the study but these were not confined to any single test group and were not related to the test material. The signs included soft stools/diarrhea, hypothermia, red ocular discharge and body weight loss. A total of 3 animals died.
Skin reactions following the challenge application were as follows:

Test group: Very slight erythema in 3/9 animals
Naive control: Very slight erythema in 3/9 animals
Vehicle control: Very slight erythema in 1/9 animals
Positive control: Very slight to severe irritation 20/20 animals. The reaction of 19 exceeded the highest reaction observed in the naive positive control group
Naive positive control: Very slight erythema in 5/20 animals

Reliability : (1) valid without restriction

(18)

Type	:	Buehler Test
Species	:	Guinea pig
GLP	:	Yes
Test substance	:	Gas oils various, see section 1.1.1.

Result : In addition to the test described above on sample API 83-08, four other samples of gas oil were tested and found to be non-sensitizing. References to all the appropriate studies are as follows:

Sample	CAS No.	Reference
Predominantly aromatic		
API 83-07	64741-59-9	API 33-30162
API 83-08	64741-59-9	API 32-32859
Predominantly saturates		
API 81-10	64742-80-9	API 31-31414
API 83-11	64742-44-2	API 32-32857
API 81-09	64742-80-9	API 31-31352

Reliability : (1) valid without restriction

(3) (10) (11) (18) (19)

5.4 REPEATED DOSE TOXICITY

Remark : Six 28-day dermal studies and two 90-day dermal studies have been reported for gas oils. Whereas one of the 28 day studies involved daily application of test material five days each week, the other 28 day studies involved application three times weekly.
Full robust summaries are given for both of the 90-day studies.
A full robust summary is given for the 28 day study in which test material was applied daily, five times per week.
A full robust summary is also given for a 28 day study in which test material was applied three times weekly.
A summary table of the results of all the studies involving thrice weekly application of test material is also provided in this section.

Species	:	Rabbit
Sex	:	Male/female
Strain	:	New Zealand white
Route of admin.	:	Dermal
Exposure period	:	6 Hours
Frequency of treatm.	:	Once a day, three times weekly for 4 weeks
Doses	:	250, 500 & 1000 mg/kg
Control group	:	Yes, concurrent no treatment
Year	:	1985
GLP	:	Yes
Test substance	:	API 83-07 (predominantly aromatic) see section 1.1.1.

Method : Approximately 24 hours before administration of the test material, the dorsal skin was clipped free of hair. The exposure site was subsequently reclipped as necessary throughout the study. Immediately prior to the first application of test material, the skin was examined and scored for irritation using the standard Draize scoring procedure for erythema and edema.

Undiluted test material was applied to the skin of 5 male and 5 female rabbits at dose levels of 250, 500 and 1000 mg/kg. The males weighed between 2.7 and 3.2 kg and the females weighed between 2.6 and 3.3 kg at the start of the study. The treated site was covered with gauze and an occlusive dressing and these remained in place for a 6 hours exposure period. At the end of the exposure, the dressings were removed and any residual test material was removed by wiping with a dry gauze pad. Dosing was carried out 3 times weekly for 4 weeks i.e. until 12 doses had been applied. A group of 5 male and 5 female animals served as sham controls.
Checks were made twice daily for mortality, moribundity and for signs of pharmacologic and toxic effects. Body weights were recorded immediately before the first dose was applied and weekly thereafter. Before each application of test material the skin was examined and scored for erythema and edema as before. Animals that died during the study were subjected to a complete gross necropsy. At the end of the dosing period surviving animals were sacrificed and a complete gross necropsy was performed.
At termination, blood samples were taken from every animal and determinations were made of: RBC count, WBC count, differential WBC count, hemoglobin concentration, hematocrit, glucose, blood urea nitrogen, alkaline phosphatase, SGOT, SGPT and total protein.
The heart, liver, spleen, kidneys, adrenals, thyroid (with parathyroids), pituitary, testis, ovary and brain were removed and weighed. The following tissues/organs were fixed for subsequent histopathological examination:

Heart	Sacculus rotundus	Urinary bladder
Lungs	Colon	Adipose tissue
Bronchi	Thymus	Mammary gland
Trachea	Spleen	Brain (cerebrum,
Thyroid	Liver	cerebellum, pons)
Parathyroids	Pancreas	Pituitary
Cervical lymph nodes	Kidneys	Spinal cord
Salivary gland	Adrenals	Skeletal muscle
Tongue	Vagina	Sciatic nerve
Esophagus	Seminal vesicles	Skin (treated and untreated)
Stomach	Testes/ovaries	Bone
Duodenum	Epididymides	Bone marrow
Jejunum	Prostate/uterus	Eyes
Ileum	Mesenteric lymph nodes	Gross lesions

Statistical analyses:
Body weight, clinical chemistry and absolute and relative organ weight data were compared to control group data of the same sex using a two-tailed Student's t-test at the 5% probability level.

Result : A high dose and a low dose male died during the study and a low dose female was sacrificed in extremis. None of these deaths were considered to be treatment-related. There were no treatment-related clinical signs during the study. The animals weighed approximately 3 kg at the start of the study and mean weight gains (kg) over the course of the study are shown below:

	Mean weight gain (kg)	
Dose level	**Males**	**Females**
Control	0.4	0.2
250 mg/kg	0.2	0.5
500 mg/kg	0.1	0.1
1000 mg/kg	0.1	0.0

Although treatment seemed to have a slight effect on weight gain, the report concluded that such differences (0.1 or 0.2 kg) are quite common in rabbits of the age used in the study and that no definitive conclusions could be drawn.

Treatment caused skin irritation to varying degrees depending on dose level. A mean irritation score (mean of the sum of all irritation scores) was calculated for each group and the results are tabulated below.

Group/sex	Mean irritation Score	Classification
Control (M)	0.0	Non irritant
Control (F)	0.0	Non irritant
250 mg/kg (M)	2.3	Moderate irritant
250 mg/kg (F)	2.1	Moderate irritant
500 mg/kg (M)	3.8	Moderate irritant
500 mg/kg (F)	4.8	Moderate irritant
1000 mg/kg (M)	5.5	Severe irritant
1000 mg/kg (F)	5.3	Severe irritant

There were no treatment-related effects on either the hematological or clinical chemical determinations. Minor differences found were incidental and not dose-related. There was a reduction in left ovarian weight of the high dose females (0.129 compared to 0.193 g), but in the absence of any corresponding histopathological data was deemed to be within normal biological variation. No other treatment-related effects on absolute or relative organ weights were found.
The only treatment-related findings at gross pathology were those at the treated skin site. There were no other gross findings that were considered to be related to exposure to test material. The treated skin was described as dry, reddened, flaky, cracked, fissured and/or leathery and thickening of the dermis was also frequently noted. These changes were only seen in the exposed groups and not in the controls.
Microscopic examination found moderate to severe proliferative and moderate to severe inflammatory changes present in the skin of all animals in the high dose group (except in the animal that died early). Concurrently with these changes there was an increased granulopoiesis of the bone marrow. This was attributed to stress of the animals due to the severe skin irritation.
Two male rabbits in the high dose group had multifocal areas of hypoplasia of some of the seminiferous tubules. These testicular changes were considered to be secondary to the skin changes and not a direct effect of the test material.

Reliability : (1) valid without restriction

(14)

Species : Rabbit
Strain : New Zealand white
Route of admin. : Dermal
Exposure period : Up to 4 weeks

Result : The following table summarizes the results of all five 28-day studies dermal studies in rabbits.

Sample API No.	Dose*	Skin Irr'n**	Growth rate/mortality	API report
Predominantly aromatics				
83-07	250	mod.	2/10 died	
	500	mod.	no effect	32-32751
	1000	sev.	1/10 died	
83-08	200	mod.	2/10 died	
	1000	sev.	weight loss	32-32753
	2000	sev.	2/10 died, weight loss	
Predominantly saturates				
83-11	200	sl.	no effect	
	1000	mod.	no effect	32-32747
	2000	mod.	weight loss in females	
81-09	200	sl.	no effect	
	1000	mod.	no effect	30-32298
	2000	mod. to sev.	no effect	
81-10	200	sl.	no effect	
	1000	mod.	no effect	30-32296
	2000	sev.	6/10 died	

* Dose given in mg/kg/day
** sl = slight
mod = moderate
sev = severe

(6) (7) (13) (14) (15)

Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period : 28 days
Frequency of treatm. : Once daily, five days each week for 4 weeks
Doses : 0.05, 0.25 & 1.0 ml/kg/day
Control group : Yes
Year : 1992
GLP : Yes
Test substance : Gas oil sample F-188 (predominantly saturate)
Sample F-188 is a gas oil containing
86% saturates
14% aromatics

Method : Three groups of ten male and ten female young adult Sprague-Dawley rats were administered test material to the shorn dorsal skin once daily, five days per week for four weeks at doses of 0.05, 0.25 or 1 ml/kg/day. The applied material was covered with an occlusive patch for six hours. A further group of ten male and ten females served as sham-treated controls. The animals were observed twice daily for clinical signs of toxicity. Dermal irritation at the application site was assessed daily prior to the next application of test material. An assessment of dermal irritation was also made 24 hours after the final application, just prior to necropsy.
Body weights were recorded three times weekly and just prior to necropsy.
At necropsy, a blood sample was taken for the following hematological and clinical chemical determinations:
Hematology

- Erythrocyte count
- Total leucocyte count
- Differential leucocyte count
- Hemoglobin
- Hematocrit
- Platelet count
- Mean corpuscular volume (MCV)

Clinical chemistry

Sodium	Glucose
Potassium	Blood urea nitrogen
Alkaline phosphatase	SGOT
SGPT	Chloride
Calcium	Phosphorus
Total protein	Creatinine
Cholesterol	Triglyceride
Albumin	Globulin (calculated)
A/G ratio (calculated)	

The following organs were weighed:

Liver
Kidneys (2)
Testes (2)/Ovaries (2)
Brain
Adrenal glands (2)

The following tissues were taken, were fixed and prepared for microscopic examination.

Accessory genital organs	Lungs with trachea
prostate	Mammary glands
seminal vesicles	Pancreas
epididymis	Peripheral nerve
Adrenal glands (2)	Pituitary
Aorta	Rectum
Brain	Salivary gland
cerebrum	Skeletal muscle (thigh)
cerebellum	Skin
pons	(treated and untreated)
Cecum	Spinal cord (cervical, mid thoracic & lumbar)
Cervical lymph nodes	Colon
Duodenum	Esophagus
Spleen	Eyes (2)

Sternum with bone marrow	Femur with articular surface
Stomach	Gross lesions and masses
Testes/ovaries (2)	Heart
Thyroid gland with	Ileum
parathyroids	Jejunum
Thymus	Kidneys (2)
Urinary bladder	Liver
Uterus	Vagina

Histopathology was done on the sham treated control group and the high dose group animals only.

Result : There were no mortalities during the study and there were no dose-related clinical observations except for the occurrence of skin irritation in all treatment groups.
Body weights were unaffected by exposure to the test material. No dermal irritation was observed in the sham-treated controls. Very slight dermal irritation was noted in both males and females in the lowest dose group (0.05 ml/kg/day). This consisted of slightly dried skin, very slight erythema and slight eschar.
In the 0.25 ml/kg/day group, irritation was slightly more and consisted of: very slight to slight (primarily very slight) erythema, slight to extreme (primarily slight to moderate) eschar and slight to moderate dried skin. Slight edema was seen in one female with slight ulceration noted in two females.
Moderate dermal irritation was seen in both males and females in the highest dose group (1 ml/kg/day). This consisted of very slight to severe erythema, slight to extreme eschar, slight to extreme dried skin, slight to extreme ulceration and very slight to slight edema.
At gross necropsy, the only treatment-related finding was skin irritation.

There were no treatment-related hematological findings.
Although differences were found in the globulin concentration and the A/G ratio they were not considered to be significant since they were not associated with any other findings and also fell within the range of normal values for Sprague-Dawley rats of the same age.
Organ weights, organ weight/body weight ratios and organ/brain weight ratios were unaffected by exposure to the test material.

The only tissues examined histologically were those from the controls and high dose group animals.
Apart from findings in the skin, there were no other treatment-related findings. The skin findings consisted of acanthosis, epidermal crusting, erosion, fibrosis, hyperkeratosis and ulceration. The incidence and severity of the lesions was greater in the high dose group than in the control group.

It was concluded that the NOAEL with respect to skin irritation was less than 0.05 ml/kg/day and the NOAEL for systemic toxicity was 1 ml/kg/day.

Reliability : (1) valid without restriction

(60)

Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period : 13 Weeks
Frequency of treatm. : 5 Days each week for 13 weeks
Doses : 8, 25, 125, 500 & 1250 mg/kg

Control group	:	Yes
Year	:	1985
GLP	:	Yes
Test substance	:	Mobil Light Cycle Oil (LCO) (predominantly aromatic) See section 1.1.1.
Method	:	Light Cycle Oil (MEHSL Sample No. 8281), was applied to the clipped backs of groups of 10 male and 10 female Sprague-Dawley rats (males approx 230g and females approx 180 g at start of study). Application was 5 days/week for 13 weeks at doses of 8, 25, 125, or 500 mg/kg/day, and to an additional group of 10 males and 10 females for 2 weeks at a dose level of 1250 mg/kg/day. Males weighed approximately 240 g and females weighed approximately 180 g at the beginning of the study. The rats were fitted with cardboard Elizabethan collars to minimize ingestion of the test article which was applied to the skin undiluted and not covered with any dressing. A similar group of 10 male and 10 female Sprague-Dawley rats served as controls. They were treated the same as the test animals, except that no material was applied to their skin. Assessment for toxic response included daily clinical observations and weekly body weight measurements. After 13 weeks the rats were killed and were carefully examined for grossly visible changes. Selected organs were weighed. Histopathological evaluation was limited to the small intestine, gonads, liver, kidneys, treated skin, spleen, stomach, thymus and urinary bladder for the control animals and those given 500 mg/kg test material per day. Blood samples were taken at 4 and 13 weeks for hematologic and serum chemical analyses. The statistical methods that were used were not specified in the report.
Result	:	Administration of test material at 500 and 1250 mg/kg/day resulted in systemic toxicity. At the high dose males and females gained less weight than controls whereas at 500 mg/kg/day only the males were affected. The animals in the high dose group looked so poorly that they were killed at the end of the second week of treatment. The females at 500 mg/kg/day and the males at 125 mg/kg/day had slightly lower body weights than the respective controls but the investigators were not clear as to whether this was a compound-related effect. LCO also caused marked, persistent effects at the site of application; severe erythema and edema with visibly thick, stiffened skin were observed. Microscopic examination of the skin from the 500 mg/kg/day group revealed moderate chronic inflammatory changes of the skin and hair follicles. The thymus was the most affected organ. In the 500 mg/kg/day group, thymus size (visually judged) and weight for both males and females was smaller than controls. Males were more affected than females. At the 125 mg/kg/day dose level only, males had slightly reduced thymus weights. The investigators judged these thymus weight differences to be attributable to a depletion of lymphocytes within the thymus. The livers of the male and female 500 mg/kg/day groups were slightly larger than controls. Also the males at this dose level had more fat in the liver cells than the controls. The investigators concluded that the effects on body and thymus weights followed a dose-response pattern. They judged the NOAEL for males and females to be 25 and 125 mg/kg/day respectively.
Reliability	:	(4) not assignable The report did not contain tables of actual data. Comments in the results section in this robust summary are taken directly from the text of the report.

The hematological and clinical chemical parameters that were measured are not specified in the report. Since raw data are not presented in the publication, it is not possible to assign a reliability to this study. However, it does provide sufficient information for a conclusion to be made on the repeat-dose toxicity of the test material.

(56)

Species	:	Rat
Sex	:	Male/female
Strain	:	Sprague-Dawley
Route of admin.	:	Dermal
Exposure period	:	13 weeks
Frequency of treatm.	:	Once a day, 5 days each week for 13 weeks
Post exposure period	:	
Doses	:	30, 125, 500 & 2000 mg/kg
Control group	:	Yes
NOAEL	:	< 30 mg/kg
Year	:	1991
GLP	:	No data
Test substance	:	Coker light gas oil (Predominantly saturate): See section 1.1.1.

Method :

Test material was applied to the shorn skin of groups of 10 male and 10 female rats (approximately 40 days old) at dose levels of 30, 125, 500 and 2000 mg/kg. A group of 10 rats of each sex served as controls.
The test material was applied each day, 5 days each week for 13 weeks except for the 500 and 2000 mg/kg groups that were sacrificed in weeks 9 and 2 respectively. All rats were fitted with Elizabethan collars to prevent ingestion of test material. The collars were removed at the end of each week and any residual test material removed from the skin by wiping. Collars were replaced on Mondays before commencement of dosing for the next week.
Body weights were recorded before application of the first dose of test material and weekly thereafter. There were daily observations for clinical signs of toxicity and an assessment and scoring of the treated skin site was made once each week according to the standard Draize scale. Urine samples were collected during weeks 5 and 13 for urinalysis (pH, specific gravity, bilirubin, urobilinogen, blood, protein, glucose and ketone). Blood samples were taken at the end of the study for the determination of the following clinical chemical and hematological parameters.

<u>Hematology</u>

Red cell count	Hemoglobin
Hematocrit	White cell count
Platelet count	

<u>Clinical chemistry</u>

Sorbitol dehydrogenase	Cholesterol
Alanine aminotransferase	Urea nitrogen
Aspartate aminotransferase	Total protein
Alkaline phosphatase	albumin (A)
Bilirubin	Triglycerides
Inorganic phosphorus	Creatinine
Glucose	Uric acid
Sodium	Potassium
Chloride	Calcium

Globulin(G) and A/G ratios were calculated

All animals surviving to the end of the study were sacrificed and necropsied. The following organs were weighed:

Adrenals	Heart	Spleen
Brain	Kidneys	Thymus
Liver	Ovaries	Uterus
Prostate	Epididymides	Testes

The following tissues/organs were removed from control group and high dose group animals and were fixed for subsequent histopathological examination.

Adrenals (both)	Ovaries (both)
Bone and marrow (sternum)	Pancreas (head)
Brain (3 sections)	Salivary gland (submaxillary)
Eye (left & optic nerve)	Skin (treated 2 sections)
Heart	Spleen
Colon	Stomach (squamous & glandular)
Duodenum	Thymus (both lobes)
Kidneys (both)	Thyroid (both lobes)
Liver (2 lobes)	Urinary bladder
Lung (left lobe)	Uterus (body & horns)
Skeletal muscle (thigh)	Gross lesions
Peripheral nerve (sciatic)	

In addition the following tissues/organs were removed, fixed and examined microscopically from the mid and low dose animals:

Adrenals	Sternum (bone and marrow)
Kidneys (both)	Liver (2 lobes)
Lung	Skin (2 sections plus any gross lesions)
Thymus	Gross lesions.

At the end of the study the epididymides and testes from the male rats in the control and 125 mg/kg groups were removed. Prior to sample preparation for testis examination, the tunica albuginea and corresponding blood vessels were removed and discarded before the remaining testicular parenchyma and cauda epididymis were weighed. Testes were prepared for spermatid count and epididymides were prepared for spermatozoa count and a morphological assessment was made of testes and epididymides.

Statistical analysis
Body weight, serum chemistry, hematology and organ weight data were analyzed by parametric methods: analysis of variance and associated F-test, followed by Tukey's multiple comparison test (body weight, hematology and organ weight data) or Student-Newman-Keuls multiple comparison test (serum chemistry), provided that there was statistical significance in the analysis of variance.
Differences between control and treated groups were considered statistically significant only if the probability of the differences being due to chance was less than 5% ($P<0.05$).

Result : The animals in the 2000 and 500 mg/kg groups were sacrificed during weeks 2 and 9 respectively due to severe skin irritation and moribund condition.
Skin irritation, generally severe, was seen in all treated animals. Apart from the observation of perineal staining, which was seen in all groups,

there were no remarkable clinical findings during the study.
During the weeks they were on the study, body weights of the 2000 and 500 mg/kg groups for both sexes was significantly less than controls. Body weights of males in the 125 mg/kg group were less than controls from day 36 onwards and although there were three occasions on which the male body weights in the 125 mg/kg group were also reduced there were no other effects on growth rates.
Results of urinalysis were in general unaffected by exposure to the test material.
Statistically significant differences between treated and control clinical chemical analyses at 13 weeks are summarized below. Only the results at 13 weeks are shown, the 5 week results are NOT included.

Parameter % change compared to control

	30 mg/kg		125 mg/kg	
	Male	Female	Male	Female
Uric acid	-	-	-	-
Glucose	-12%	-	-17%	-20%
Urea Nitrogen	-	-	-	+21.6%
AST	-33%	-	-31%	-
ALT	-	-	-	-
Alk. Phos.	-	+30%	-	+35%
Creatinine	-	-	-	-
Cholesterol	-	-	-	-
Triglycerides	-	-	-	-
Total protein	-	-	-	-
Bilirubin	-	-	-	-
Albumin	-	-	-	-
Calcium	-	-5%	-	-6%
Phosphorus	-	-	-	-
Sodium	-	-	-	-
Potassium	-	-	-	-
Chloride	-	-	-2%	-
A/G ratio	-	-	-	-
Globulin	-	-	-	-
SDH	-	+40%	-	-30%

At 13 weeks the hematological parameters affected were:

* an increase in WBCs and the number of segmented neutrophils in the high dose males and females
* an increase in lymphocytes in the 125 mg/kg group of both sexes and the 30 mg/kg group females.

No effects were found in any of the sperm evaluations that were made.
The only effect noted in the absolute and relative organ weight data of the 30 mg/kg group was a reduction of approximately 10% in the absolute thymus weight in males.

In the 125 mg/kg group there were more differences noted in organ weights and these are tabulated below as percentage change. The authors judged that the decreases in organ weights were probably due to the reduced body weights of the animals. However, they also judged that the effect was probably compound related for those organs for which there were differences in organ/body weight ratio.

A = Absolute weight, R= Organ/body weight ratio

Organ		Male	Female
Adrenals	(A)	-	-
	(R)	+29%	-
Brain	(A)	-	-
	(R)	+11%	-
Epididymides	(A)	-	-
	(R)	+11%	-
Heart	(A)	-	-
	(R)	-	+6%
Kidneys	(A)	-	-
	(R)	+8%	+8%
Liver	(A)	-	-
	(R)	+13%	+22%
Prostate	(A)	-	
	(R)	+17%	
Spleen	(A)	-	-
	(R)	+19%	+19%
Testes	(A)	-	
	(R)	+11%	
Thymus	(A)	-34%	-23%
	(R)	-25%	-
Uterus	(A)		-
	(R)		-
Ovaries	(A)		-
	(R)		-

The primary treatment-related changes observed at histopathological examination were severe skin irritation and slight effects on bone marrow and kidneys.

The bone marrow effects included:

* at 2000 mg/kg a severe reduction in erythropoietic cells and megakaryocytes
* at 2000, 500 and 125 mg/kg megakaryocyte changes characterized by larger, vacuolated, and/or darkened nuclei or clumped cell effects

The kidney effects included:

* Basophilia in the tubular cortex, predominantly in males. Focal inflammation, dilation of ducts in the medulla and tubules in the cortex.

Changes were also seen in the adrenals, liver, lungs, draining lymph nodes, prostate, seminal vesicles, spleen, thymus and uterus. However, these changes were considered to be secondary effects probably due to reduced weight gain, treatment-related skin injury, slight septicemia and stress.

Reliability : (1) valid without restriction

(59)

Type : Sub-chronic
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : 6 hours
Frequency of treatm. : Daily, five days each week for four consecutive weeks

Doses : Nominal 25 mg/m³
Control group : Yes
Year : 1986
GLP : Yes
Test substance : API 81-09 & API 81-10 [predominantly saturates]

Method : Groups of 20 male and 20 female Sprague Dawley rats (aged approximately six weeks) were exposed to a nominal concentrations of 25mg/m³ of each of two samples of hydrodesulfurized middle distillate by inhalation. Exposures were for approximately six hours each day, five days each week for four consecutive weeks. Control groups of 20 male and 20 females were exposed to filtered air.
Animals were observed twice daily for overt signs of toxicity and they underwent detailed examination once weekly. Body weights were also recorded weekly.
At study termination, the animals were killed and blood samples were taken for the following clinical chemical and hematological investigations:

Hematology	Clinical chemistry
Hematocrit	Aspartate aminotransferase
Hemoglobin	Alanine aminotransferase
Erythrocyte count	Alkaline phosphatase
MCH	Glucose
MCV	Urea nitrogen
MCHC	Total protein
Leucocyte count	
Platelet count	
Reticulocyte count	

For all rats, the following organs were weighed and the organ body weight ratios were calculated :
Heart, lung and trachea, liver, kidneys, brain, spleen, adrenals, thyroid/parathyroid, pituitary, testes and ovaries.
The following tissues were removed and preserved:

Adrenals (2)	Aorta
Bone marrow (femur)	Bone marrow smear
Brain (3 levels)	Eye with contiguous Harderian gland
Esophagus	Stomach
Duodenum	Jejunum
Ileum	Cecum
Colon	Rectum
Gonads	Ovary (2)
Kidney (2)	Testis with epididymis (2)
Heart	Liver (3 sections)
Nasal tissues	Lung & trachea (all lobes)
Abdominal lymph nodes	Thoracic lymph nodes
Mammary region lymph nodes	
Pancreas	Pituitary
Sciatic nerve	Prostate & seminal vesicle
Skeletal muscle (thigh)	Skin
Salivary gland (mandibular with submandibular lymph node)	
Spinal cord (cervical, mid thoracic & lumbar)	
Spleen	Thymic region
Thyroid/parathyroid complex	
Urinary bladder	Uterus (2 horns & cervix)
Vagina	

On all rats the following tissues were examined microscopically:
Adrenal (2), brain (3 levels: fore, mid & hind), bronchi, esophagus, eye (2), heart, kidney (2), liver, lungs (2), lymph ode (mediastinal), ovary (2), pancreas, pituitary, prostate, salivary gland, skin, spleen, stomach, testis (2), thymus, thyroid/parathyroid, trachea, urinary bladder, uterus, all gross lesions.

Statistical analysis
Body weight, hematology, clinical chemistry and organ weight data were analyzed by analysis of variance and Bartlett's test.
Treatment groups were compared to control by sex, using the appropriate t-statistic.
Data containing inequalities or where group variances were heterogeneous were compared using a non-parametric approach, by transforming the data into ranks prior to analysis as described by Conover and Iman.

Result : There were no treatment-related clinical observations during exposure, nor were there any effects on body weight.
Although there were some minor clinical chemical differences, these were considered to be unrelated to treatment.
The only noteworthy effect was an increase in leukocyte counts in males and females (29 % & 31 % respectively) exposed to sample API 81-10.
There were no macroscopic observations at necropsy.

Although there were some organ weight/relative organ weight differences, they were not considered relevant in the absence of any related microscopic pathology.

Microscopic tissue changes were confined to the nasal tissues of males and females exposed to API 81-09.
The changes consisted of subacute inflammation (Rhinitis) of the respiratory mucosa. The incidence and severity are shown in the following table.

	Male		Female	
	Control	**81-09**	**Control**	**81-09**
No. examined	20	20	20	20
Inflammation				
trace	0	7	0	3
mild	0	10	0	12

No other treatment-related changes were observed in any treatment group.

Test condition : Atmospheres were generated by atomizing the test material into an atomization chamber. The resulting vapors/aerosols were directed to the chamber inlet where dilution with chamber ventilation air reduced the concentration to the desired level.
Nominal concentrations were calculated from test material use rates.
Actual concentrations were determined by standard gravimetric techniques.
Aerosol particle size was also determined.
The nominal and actual concentrations for the study were:

		Exposure concentration (mg/m³)			
Sample	**Desired conc. mg/m³**	**Nominal**		**Actual**	
		Mean	**SD**	**Mean**	**SD**
API 81-09	25	127	16.8	23	4.41
API 81-10	25	160	68.1	24	9.73

Particle size determinations showed the following:

	Week	EAD* (mcm)	GSD**
API 81-09	1	3.6	2.03
	3	3.5	2.19
API 81-10	1	3.2	2.10
	3	3.3	2.11

* EAD = Equivalent Aerodynamic Diameter
** GSD = Geometric standard deviation

Reliability : (1) valid without restriction

(39)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Modified Ames bioassay
System of testing : S. Typhimurium Strain TA98
Test concentration : 1 to 60 µl
Metabolic activation : With and without
Year : 1991
GLP : No data
Test substance : 14 DGMK samples of gas oil (DMK 1-14 inc) See section 1.1.1.

Method : A modified Salmonella mutagenicity assay was performed at the Mobil Environmental and Health Science Laboratory. The technique that was used has been described fully elsewhere (Blackburn et al 1984 & 1986).

The middle distillate samples (2 ml) were dissolved in cyclohexane and the solution was then extracted with DMSO (10 ml). These extracts were tested in Salmonella typhimurium strain TA98.
The concentrations of DMSO extract used were: 60, 50, 40, 30, 20 15, 10 and 5 µl/60 µl. Extra concentrations were used for some assays.
Positive controls were 2.0 µg 2-aminoanthracene, 10.0 µg benzo(a)pyrene and 25 µg 2-nitrofluorene in 50 µl DMSO per bacterial plate.
Metabolic activation was accomplished by using an eight-fold higher concentration of the liver S9 fraction obtained from Arachlor-induced Syrian Hamsters rather than rats.
NADP cofactor was also increased from the normal 4 to 8 mM.

Result : A mutagenicity index (MI) was calculated, which represented the slope of the dose response curve for each of the samples.
Previous studies have established that materials with an MI of less than or equal to 1.0 have not been associated with a tumorigenic response in skin painting bioassays, whereas those materials with an MI greater than 1.0 have been associated with a tumorigenic response.
The MIs for the 14 middle distillate samples were:

Sample	PAC content*	Mutagenicity Index	Aromatic content
Predominantly aromatic			
10	8.3	9.3	59.8
7	11.3	9	55.1
14	5.4	7.6	52.4
Predominantly saturates			
6**	4.2	4	48.3
13**	2.4	2.3	46.9

12**	3.7	3.1	42.6
9	4	4	42.5
5	4.7	1.4	33.9
2**	4	1.3	32.6
4	1.4	0.7	29.3
3	2.8	1	28.1
8	4	2.1	27
1	1.8	0.8	23.6
11**	1	0.7	21

* Total weight % PAC
** Sample contains olefins

Test substance : A total of 24 samples were tested in this study as follows.

Straight run gas oils	Samples 1 - 5 inc.
Cracked gas oils	Samples 6 - 14 inc.
Light fuel oil	Samples 15 - 21 inc
Diesel fuel	Samples 22 - 24

This summary only includes the results on the gas oils (samples 1-14 inc). Results of the studies on the fuels (samples 15-24 inc) are summarized in the robust summaries on distillate fuels.

Reliability : (1) valid without restriction

(41) (42) (46)

Type : Mouse lymphoma assay
System of testing : Forward mutation assay using cell line L5178Y TK+/-
Test concentration : 5 to 80 nl/ml without activation and 2.5 to 30 nl/ml with activation
Metabolic activation : With and without
Method : OECD Guide-line 476
Year : 1985
GLP : Yes
Test substance : API 83-07 (See section 1.1.1.)

Method : The test material was dissolved in ethanol for this assay.
Two positive control substances were used viz ethyl methane sulphonate (EMS) at concentrations of 0.25 & 0.4 µl/ml and 3-methylcholanthrene (MCA) at concentrations of 2.5 & 4.0 µg/ml.

A cytotoxicity study carried out prior to the mutagenicity assay established that the sample was highly toxic at 62.5 nl/ml without activation and lethal at the same concentration in the presence of metabolic activation.

For the mutation assay the lymphoma cells were exposed for 4 hours to test material at dose levels up to 120 nl/ml without activation and up to 60 nl/ml with S-9 activation.
After exposure to the test material, the cells were allowed to recover for 2 days and then cultures were selected for cloning and mutant selection. Plates containing colonies of selected cells were incubated for 10 to 14 days after which they were scored for total number of colonies per plate. A mutation frequency was then determined.

Result : After the 2 day recovery period, seven non-activated cultures and seven S-9 activated cultures were cloned based on their degree of toxicity. The mutant frequencies and the percentage total growth at each of the test concentrations is summarized in the following table.

Concentration (nl/ml)	Mutant frequency	Relative growth %
Non-Activated		
5	34	34.2
15	19.1	67.5
30	32.2	81.1
40	21.9	153.8
50	18.9	55.5
60	41.8	33.8
80	Excessive toxicity, treatment not cloned	
Solvent 1	33.5	100
Solvent 2	19.2	100
Solvent 3	30.1	100
EMS 0.25 µl/ml	426.1	38.2
EMS 0.4 µl/ml	570.2	58.3
S-9 Activated		
2.5	52.5	66.2
5	67	74.8
10	158.5	64.8
15	198.5	22.3
20	207.6	12.8
25	255.3	6.7
30	Excessive toxicity, treatment not cloned	
Solvent 1	48	100
Solvent 2	56.5	100
Solvent 3	55.7	100
MCA 2.5 µg/ml	209.3	78
MCA 4 µg/ml	411.2	33.1

According to the criteria used by the authors to judge the activity of the test material, the sample produced a positive response in the presence of S-9 activation but was not mutagenic in the absence of activation.

Reliability : (1) valid without restriction

(23)

Type : Mouse lymphoma assay
Test substance : other TS: various gas oils (see section 1.1.1.)

Result : Mouse Lymphoma assays have been carried out on 6 different PI samples, including API 83-07. Te results for all API samples are summarized below.

API sample	Result
Predominantly aromatic samples	
83-07	Positive with S9 activation Negative without S9 activation (Ref API 32-32167)
83-08	Positive with and without S9 activation (Ref API 32-31709)
81-10 (Aromatic fraction)	Negative with and without S9 activation (Ref API 34-32644)

Predominantly saturates

83-11	Laboratory 1 (Ref API 32-32166) Positive with S9 activation Negative without S9 activation
	Laboratory 2 (Ref API 32-31768) Positive with and without S9 activation
81-09	Positive only at high toxicity without S9 activation (Ref API 32-30965)
81-10	Test 1 (Ref API 32-30535) Positive at moderate to high toxicity with S9 activation only
	Test 2 (Ref API 33-31224) Weakly positive with and without S9 activation
	Test 3 (Ref 34-32643) Positive with S9 activation only
	Saturates fraction (Ref API 34-32645) Negative with and without S9 activation

(12) (20) (21) (22) (23) (24) (28) (31) (32) (33)

Type : Sister chromatid exchange assay
System of testing : Chinese Hamster Ovary Cells (CHO)
Test concentration : 0.1 to 1000 µg/ml
Metabolic activation : With and without
Result : Ambiguous
Year : 1988
GLP : Yes
Test substance : API 83-07 (See section 1.1.1.)

Method : A cytotoxicity study was performed in order to select dose levels for the SCE assay.
For the SCE assay CHO cells were seeded in duplicate for each treatment condition and were incubated at 37°C in a humidified atmosphere for 16 to 24 hours. Treatment was carried out by refeeding two complete sets of flasks with complete medium for the non activation study or with S-9 reaction mixture for the activated study to which was added 50 µl of dosing solution of test control or article in solvent or solvent alone. In the non-activation study the cells were exposed for about 26 hours. Two hours after exposure 0.01 mM BrdUrd was added to the treatment medium. At the end of the treatment period, the treatment medium was removed, the cells rinsed and then exposed to colcemid (0.1 µg/ml) for a further 2 hours. In the activation study exposure was for 2 hours. After the exposure period, the treatment medium was removed, the cells were washed re-fed with medium containing BrdUrd and then incubated for a further 26 hours. Colcemid was added for the last 2 hours of incubation.

For activated and non-activated assays metaphase cells were harvested 2 hours after addition of colcemid. Cells were collected and fixed and stored until slides were prepared.

Slides were coded and scored without regard to treatment group. Only cells with 20 ± 2 centromeres were selected for evaluation of SCEs. A total

of 4 doses were scored including the highest test article dose where sufficient second-division metaphase cells wee available. SCEs were scored in 25 cells from each duplicate culture to make up a total of 50 cells per treatment. The percentage of cells in first (M1), second (M2) or third division (M3) metaphase was also recorded for a total of 100 metaphase cells scored. TEM was used as positive control at a concentration of 0.025 µg/ml. in the non-activated assay. In the activated assay CP was used as a positive control at a concentration of 2.5 µg/ml.

A test was deemed valid if the mean SCE/cell in the untreated control did not exceed 13 and the mean SCE/cell for the positive control was at least double that of the negative control.

A test material is considered positive if it induces a doubling in SCE frequency over the solvent control at a minimum of three consecutive dose levels or if a dose responsive and statistically significant increase is observed over a minimum of 3 dose levels.

Remark : In a separate Sister Chromatid Exchange assay, Sample API 81-10 (sample containing predominantly saturates) was negative without S9 activation. When tested with S9 activation, the result was equivocal and there was no dose relationship. (Ref API 35-32433)

Result : In the non-activation assay the two highest dose levels could not be evaluated due to severe cell delay and the absence of scorable second-division metaphase cells. Since only two dose levels were scorable and they were both significantly greater than the solvent controls. A repeat study was conducted but at dose levels of 2.5, 5, 10, 20 and 30 µg/ml. However, only the four highest doses were scored for SCEs. The results of both studies are summarized below.
Statistically significant differences are indicated:
* P less than or equal to 0.05 by Student's t test
** P less than or equal to 0.01 by Student's t test

Treatment/Replicate		SCEs/ chromosome	Group mean SCEs/cell
Non-activation assay (FIRST study)			
Untreated cells	A	0.51	
	B	0.61	11.00 ±3.9
Acetone	A	0.62	
	B	0.58	11.82 ±2.76
API 83-07			
10 µg/ml	A	0.61	
	B	0.78	13.68 ±4.43**
20 µg/ml	A	0.65	
	B	0.7	13.26 ±3.95*
40 µg/ml	A	Not determined	
	B	Not reported	
80 µg/ml	A	Not determined	
	B	Not determined	
TEM	A	3.06	
	B	3.49	63.86±13.07**

Non-activation assay (REPEAT study)

Untreated cells	A	0.55	
	B	0.55	10.74 +/-3.02
Acetone	A	0.57	
	B	0.59	11.42 +/-3.49
API 83-07			
5 µg/ml	A	0.60	
	B	0.49	10.70 +/-3.33
10 µg/ml	A	0.57	
	B	0.60	11.52 +/-3.56
20 µg/ml	A	0.63	
	B	0.57	11.76 +/-2.79
30 µg/ml	A	0.75	
	B	0.63	13.40 +/-4.84*
TEM	A	1.60	
	B	1.57	31.00 +/-6.38**

In the repeat study the frequency of SCEs was significantly greater than the solvent controls in the high dose only.

The results from the activation assay were as follows:

Activation assay

Untreated cells	A	0.62	
	B	0.62	12.20 +/-3.99
Solvent	A	0.67	
	B	0.67	13.16 +/-3.88
API 83-07			
10 µg/ml	A	0.76	
	B	0.76	14.86 +/-4.60*
20 µg/ml	A	0.65	
	B	0.71	13.44 +/-3.36
40 µg/ml	A	0.67	
	B	0.80	14.60 +/-4.13*
40 µg/ml	A	0.82	
	B	0.77	15.58 +/-4.34**
80 µg/ml	A	0.82	
	B	0.77	15.58 +/-6.34**
Cyclophosphamide	A	1.57	
	B	1.63	31.56 +/-6.34**

In this assay the increases in frequency of SCEs at all but one dose level were significantly greater than that for the solvent controls.

Conclusion : The authors concluded: The positive and negative controls fulfilled the requirements for a valid test.

Under the conditions described in the report, API 83-07 did induce an increase in SCEs in CHO cells at one or two concentrations in two independent studies when tested in the absence of exogenous activation and at three non-consecutive concentrations when tested in the S-9 activated system in a single study. In the non-activated test system, activity at a single concentration was not reproducible. Because the increase in SCEs above the spontaneous background level had no clear dose-response, API 83-07 was concluded to be equivocal in this test

system.

Reliability : (2) valid with restrictions

(35) (36)

Remark : In a separate Sister Chromatid Exchange assay, Sample API 81-10 (sample containing predominantly saturates) was negative without S9 activation. When tested with S9 activation, the result was equivocal and there was no dose relationship. (Ref API 35-32433)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : i.p.
Exposure period : 6, 24 and 48 hours
Doses : 2, 0.67 & 0.2 g/kg
Result : Negative
Method : OECD Guide-line 475 "Genetic Toxicology: In vivo Mammalian Bone Marrow Cytogenetic Test - Chromosomal Analysis"
Year : 1986
GLP : Yes
Test substance : API 83-07 (Sample consisting predominantly of aromatics (See section 1.1.1.)

Method : Undiluted test material was given intraperitoneally to groups of 15 rats of each sex at three different dose levels (0.2, 0.67 & 2.0 g/kg). A group of 15 rats of each sex, serving as negative controls, were given deionized water. A group of 5 animals of each sex, used as positive controls was dosed with 1.0 mg/kg triethylenemelamine (TEM) and these animals were killed 24 hours afterwards. Three hours prior to being killed the rats were given a single i.p dose of colchicine (4 mg/kg).
For each dose level of test material and the negative controls 5 rats of each sex were killed 6, 24 and 48 hours after dosing.
Immediately following sacrifice bone marrow was aspirated from the tibiae. The marrow was washed and the cells were fixed before being spread on slides for examination (routinely 50 spreads for each animal). Slides were stained and scored for chromatid and chromosome gaps and breaks, fragments, structural rearrangements and ploidy. A mitotic index based on at least 500 cells was recorded. The index was calculated by scoring the number of cells in mitosis per 500 cells on each slide read.

The data on chromosomal aberrations for the treated animals was compared to that for the negative controls.
The criteria used in assessing the result are described in the results section.

Result : Immediately after dosing all animals in the 2 g/kg group were lethargic. There were some mortalities in the high dose group and where possible they were replaced. Throughout the study lethargy was observed in the high dose group animals but no toxic signs were seen in any of the other dose groups.
A summary of the chromosomal results is given in the following table.

Group	Time	Frequency of aberrations	% cells 1+ aberrations Str*	% cells 2+ aberrations Num**	Mitotic index
Male					
-ve control					
6	0	.024	0	0	4.1
24	.004	.016	.4	0	2.8
48	.01	.02	1	0	3.5
+ve control (TEM @1.0 mg/kg)					
24	>1.17	.1	29	18.8	1.3
Test material					
0.2 6	.015	.005	1.5	0	2.8
24	0	.016	0	0	4.3
48	0	.02	0	0	3.6
0.67 6	0	.04	0	0	5.9
24	>.02	.03	2	.4	4.1
48	0	.01	0	0	4.5
2.0 6	0	.028	0	0	3.8
24	.01	.01	1	0	4.7
48	0	.02	0	0	3.6
Female					
-ve control					
6	.01	.01	1	0	4.2
24	.012	.024	1.2	0	2.6
48	0	.036	0	0	4.2
+ve control (TEM @1.0 mg/kg)					
24	>4.199	0	59.9	54.5	.2
Test material					
0.2 6	0	.012	0	0	3.1
24	.004	.o4	.4	0	3.3
48	.004	.012	.4	0	2.9
0.67 6	>.016	.044	1.6	.4	4.6
24	0	.008	0	0	3.6
48	0	.016	0	0	5.5
2.0 6	0	.032	0	0	4.1
24	0	.016	0	0	4.9
48	0	.01	0	0	6.2

For simplicity only standard error values have not been shown in the above table.

The authors make the following interpretation of the results
The test material did not induce a significant increase in the percentage of aberrant cells above the controls for either sex at any of the doses or kill times. No apparent test article effects on the mitotic index were noted in any of the dose groups. The positive control (TEM) induced significant increases in the percentage of cells with structural chromosomal aberrations in the male animals (29%). The positive control also produced

a very high percentage of cells with structural chromosomal aberrations in the female dose group (59.9%). However, the data from the female positive control group could not be statistically analyzed since only 32 metaphase cells could be analyzed. The lack of analyzable metaphase cells can be attributed to bone marrow toxicity induced by the TEM.

Reliability : (1) valid without restriction

(29)

Type : Cytogenetic assay
Test substance : Gas oils various (See section 1.1.1.)

Result : A total of six bone marrow cytogenetics assays have been carried out on API gas oil samples. All of these studies have been negative.

The samples tested and the references to the original reports are:

API sample	**Reference**
Samples predominantly aromatic	
83-07	33-30929
83-08	33-30493
Samples predominantly saturates	
83-11	32-32408
	33-30930
81-09	32-30965
81-10	32-30535

(12) (16) (17) (22) (27)

Type : Sister chromatid exchange assay
Species : Mouse
Route of admin. : i.p.
Exposure period :
Doses : 340, 1700 and 3400 mg/kg
Result : Positive
Year : 1988
Test substance : API 83-07

(37)

Type : Sister chromatid exchange assay
Species : Mouse
Route of admin. : i.p.
Doses : 0.5, 2.5 and 5.0 mg/kg
Result : Negative
Year : 1988
Test substance : API 81-10

(34)

5.7 CARCINOGENICITY

Species : Mouse
Sex : Male
Strain : C3H
Route of admin. : Dermal
Exposure period : 104 weeks

Frequency of treatm. : 2, 4 or 7 days/week
Doses : Doses variable, see method
Control group : Yes, concurrent vehicle
Year : 1996
GLP : Yes
Test substance : MD-7 (See section 1.1.1.)

Method : The testing of the cracked gas oil (MD-7) was part of an overall larger study. For the purpose of this summary, only the details relating to MD-7 are presented. The test material was applied to the shorn skin of three groups of 50 male mice for 104 weeks. The concentration and dosing frequencies were adjusted to ensure that each animal received the same total weekly dose of test material irrespective of dosing frequency. The concentrations and frequencies were selected to determine the influence of skin irritation on the tumorigenic response.

The following dosing regimes were used

Group No.	Concentration of gas oil* (%)	µl/dose	Dosing frequency
9	100	50	2 times/week
10	50	50	4 times/week
11	28.5	50	7 times/week

* Mineral oil was used as diluent

A control group of 50 male mice received 35 µl mineral oil 7 days each week.

All animals were observed regularly for viability, clinical signs and a score was given for any dermal irritation that occurred. Body weights were recorded throughout the study. When they developed, dermal growths were measured and documented.

All animals were necropsied either when they died during the study or at the end of the study. The necropsy included an examination of the body, all orifices and the carcass, cranial, thoracic and abdominal cavities, including their contents.

For all animals, tissues were preserved and examined microscopically from all skin tumors, skin from treated and untreated sites and any grossly observable masses.

Result : Survival was less in the MD-7 treated groups compared to the negative controls; at the lower two concentrations (28.5 and 50 %) the difference was statistically significant. Dermal irritation occurred in the groups exposed to gas oil. The dermal irritation scores were:

Group	Range of scores	Mean dermal score
Negative control (oil)	0-0.22	0.06
100% gas oil 2X/week	0-4.0	2.4
50% gas oil 4X/week	0-4.0	1.59
28.5% gas oil 7X/week	0-1.67	0.28
Positive control (HCO)	0-2.0	0.73

There were no other treatment-related clinical findings.

Treatment-related findings at post mortem were limited to

Treatment did not have any adverse effect on body weights.

dermal irritation and were consistent with the findings of the in-life phase of the study.

Liver masses and gastrointestinal abnormalities were observed but these were found in all groups and were considered to be incidental.

Tumors developed in the positive control group (HCO) and in the MD-7 treated groups as follows:

Material	Oil	HCO	MD-7	MD-7	MD-7
Concentration			28.5%	50%	100%
No. applications/week			7	4	2
No. mice examined	50	50	50	50	50
No. mice with tumors	0	47	1	17	7
Tumor types					
Squamous cell carcinoma	0	42/73*	1	8/9*	3
Fibrosarcoma	0	0	1	4	0
Melanoma	0	0	0	1	0
Papilloma	0	37/88*	0	10/134*	

* / = No with neoplasms/actual incidence of neoplasms

Reliability : (1) valid without restriction

(49)

Species : Mouse
Sex : Male
Strain : C3H
Route of admin. : Dermal
Exposure period : 104 weeks
Frequency of treatm. : 2, 4 or 7 days/week
Doses : Variable, see methods
Control group : Yes, concurrent vehicle
Year : 1996
GLP : Yes
Test substance : MD-6 (See section 1.1.1.)
Sample MD-6 was a straight run, hydrotreated gas oil, with CAS No. 64742-46-7.
The sample contained 1.3% m/m 3-7 ring PAC
The vehicle control that was used was a Mineral oil Solvent 100 neutral low pour. CAS No. 64742-54-7

Method : The testing of the straight run gas oil was part of an overall larger study. For the purpose of this summary, only the details relating to the straight run gas oil are presented.
The test material was applied to the shorn skin of three groups of 50 male mice for 104 weeks.
The concentration and dosing frequencies were adjusted to ensure that each animal received the same total weekly dose of test material irrespective of dosing frequency. The concentrations and frequencies were selected to determine the influence of skin irritation on the tumorigenic response.

The following dosing regimes were used

Group No.	Concentration of gas oil* (%)	µl/dose	Dosing frequency
6	100	50	2 times/week
7	50	50	4 times/week
8	28.5	50	7 times/week

* Mineral oil was used as diluent
A control group of 50 male mice received 35 µl mineral oil 7 days each week.
All animals were observed regularly for viability, clinical signs and a score was given for any dermal irritation that occurred. Body weights were recorded throughout the study. When they developed, dermal growths were measured and documented. All animals were necropsied either when they died during the study or at the end of the study. the necropsy included an examination of the body, all orifices, the carcass and cranial, thoracic and abdominal cavities, including their contents. For all animals, tissues were preserved and examined microscopically from all skin tumors, skin from treated and untreated sites and any grossly observable masses.

Result : There was no significant difference between the survival of the negative controls and any of the groups receiving gas oil. Survival of the positive control group was poorer than the negative controls. Dermal irritation occurred in the groups exposed to gas oil. The dermal irritation scores were:

Group	Range of scores	Mean dermal score
Negative control	0-0.22	0.06
100% gas oil 2X/week	0-4.0	2.0
50% gas oil 4X/week	0-0.5	0.09
28.5% gas oil 7X/week	0-0.47	0.02
Positive control	0-2.0	0.73

There were no other treatment-related clinical findings. Treatment did not have any adverse effect on body weights. Treatment related findings at post mortem were limited to dermal irritation and were consistent with the findings of the in-life phase of the study.
Liver masses and gastrointestinal abnormalities were observed but these were found in all groups and were considered to be incidental.
Tumors developed in the positive control group and in two of the gas oil groups thus:

Group	Animals with skin masses	Animals with confirmed tumors
Negative control	0	0
Positive control	47	47
100% MD-6	2	4
50% MD-6	0	0
28.5% MD-6	1	1

In the group receiving undiluted MD-6 one animal had developed a basal cell carcinoma and one animal a squamous cell carcinoma. 3 mice had developed papillomas. A squamous cell carcinoma was observed in one animal receiving 28.5% MD-6 seven days a week. The tumor incidence was highest in the group in which skin irritation was greatest with no tumors developing in the middle dose group. The report suggests that the single tumor that developed in one mouse in the lowest dose group could have been spontaneous.

Reliability : (1) valid without restriction

(49)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	Rat
Sex	:	Female
Strain	:	Sprague-Dawley
Route of admin.	:	Dermal
Exposure period	:	Days 0 to 19 of gestation
Frequency of treatm.	:	Daily
Duration of test	:	Up to gestation day 20
Doses	:	25, 50, 125, 250, 500 & 1000 mg/kg
Control group	:	Yes
Year	:	1987
GLP	:	No data
Test substance	:	Mobil Light Cycle Oil (LCO) (See section 1.1.1.)

Method :

Prior to dosing, females (approximately 12 weeks old) were paired and the appearance of a vaginal plug or the presence of spermatozoa in vaginal lavage fluid was taken to indicate that mating had occurred. This was taken to be day 0 of the study. The presumed-pregnant rats were distributed into the following groups each of 10 animals.
Light cycle oil (LCO) was applied daily to the shorn dorsal skin at the dose levels shown below and for the duration indicated. The rats were fitted with collars to prevent oral ingestion of the applied material. Since it was believed that inhalation of test material could be a confounding factor a second group of controls (remote controls) were housed in an area in which they could not inhale gas oil that had been applied to other animals.
The study design is outlined as follows:

	Dose level (mg/kg/day)	Dosing days*
Group 1	0 (Remote sham control)	0-19
Group 2	0 (Proximal sham control)	0-19
Group 3	25	0-19
Group 4	50	0-19
Group 5	125	0-19
Group 6	250	0-19
Group 7	500	0-19
Group 8A	1000	0-6
Group 8B	1000	6-15

* denotes days of gestation

Observations were made daily for clinical signs. Body weights and food consumption were recorded regularly throughout the study.

Each female was sacrificed on day 20 of presumed gestation and the thoracic and abdominal cavities were examined grossly. The uterus and ovaries were removed and examined grossly. The number of corpora lutea per ovary for each rat was recorded. The ovaries of non-pregnant females were examined and then discarded. Uterus weights were also determined. The uterine contents of each pregnant rat were exposed and a record made of the number and location of all implantations.
At necropsy, blood samples were taken from all the animals and the following clinical chemical measurements were made:
Alanine aminotransferase Glucose

Albumin	Lactate dehydrogenase
Albumin/globulin ratio	Inorganic phosphorus
Alkaline phosphatase	Potassium
Aspartate aminotransferase	Sodium
Bilirubin (total)	Sorbitol dehydrogenase
Calcium	Total protein
Chloride	Triglycerides
Cholesterol	Urea nitrogen
Creatinine	Uric acid
Globulin	

Fetuses were examined and half were preserved for examination of soft tissue abnormalities, the remainder being differentially stained for skeletal examination.

Statistical analysis
Maternal biphase data, cesarean section data and fetal data were evaluated statistically by analysis of variance followed by group comparisons using Fisher's exact or Dunnet's test.
Serum chemistry data were analyzed for analysis of variance followed by comparisons using Tukey's test.

For all statistical analyses, differences between control and treated groups were considered to be significant if the probability of the difference being due to chance was less than 5% ($p < 0.05$)

Result **:** Erythema and flaking of the skin were observed in all groups exposed to LCO. Eschar, fissuring, scabbing and scar formation was observed in all but the 25 mg/kg group. Sensory Irritation was particularly severe in the 500 and 1000 mg/kg groups.
At doses higher than 25 mg/kg there was a decrease in body weight and body weight gain compared to the controls and this was accompanied by a reduction in food consumption.

There were no treatment-related findings at necropsy.
In the clinical chemical measurements there were no differences recorded for the 1000 mg/kg animals. However, cholesterol and triglycerides were increased in the 250, 500 and 1000 mg/kg groups. The dose-response was linear only for triglycerides.
Fetal body weights were reduced only in the 500 and 1000 mg/kg groups and statistical significance was achieved only in the latter group.
The number of malformations (soft tissue and skeletal) that occurred are tabulated below

Group (mg/kg)	**No. affected/ No. examined**	**No. fetuses with >1 anomaly**	**No. litters adversely affected**
0	4/135	2	2
0	137	0	0
25	1/143	1	1
50	0/111	0	0
125	0/148	0	0
250	1/136	1	1
500	2/112	2	2

No anomalies were seen in either of the 1000 mg/kg groups.

Reliability **:** (2) valid with restrictions
The report reviewed was incomplete and only a textual description (no data tables) was given of the anomalies observed.

(58)

Species	:	Rat
Sex	:	Female
Strain	:	Sprague-Dawley
Route of admin.	:	Dermal
Frequency of treatm.	:	Once daily
Duration of test	:	From days 0-20 of gestation
Doses	:	50, 150 & 500 mg/kg/day
Control group	:	Yes
NOAEL maternal tox.	:	< 50 mg/kg bw
NOAEL teratogen.	:	50 mg/kg bw
Year	:	1994
GLP	:	Yes
Test substance	:	Test material F-215 is a straight run gas oil and has the following composition: Saturates 65.4% Aromatics 34.6%

Method :

Prior to the conduct of the developmental toxicity screen a screening study for skin irritation was carried out.
The irritation screen was conducted in four female rats at dose levels of 250, 500 and 1000 mg/kg/day. The test material was applied undiluted daily for seven days.
No mortality was observed during this screening study and slight to extreme erythema, edema, eschar and dry skin were noted in all dose groups. Animals in the 250 mg/kg/day group gained body weight during the study.
Weights decreased for one of four animals in the 500 mg/kg/day group and also for three of the four animals in the highest dose group.

<u>Developmental toxicity</u>
Undiluted test material was applied once daily to the clipped skin on the backs of groups of 12 presumed-pregnant female rats, aged 12-13 weeks, on days 0 to 20 of gestation at doses of 50, 150 and 500 mg/kg/day.
Application sites were alternated (intrascapular and lumbar) to reduce skin irritation. Elizabethan collars were fitted to the rats for 6 hours after each application to reduce oral intake of test material. Any residual test material was wiped from the skin prior to collar removal.
Each animal was observed twice daily for signs of toxicity. Body weights were recorded on days 0, 4, 8, 12, 16 and 20 of gestation and food consumption was recorded for the periods 0-4, 4-8, 8-12, 12-16 and 16-20 days of gestation and days 0 and 4 of lactation.
Each litter was observed daily during days 0 through 4 of lactation for signs of toxicity and mortality.
Each female that was mated was sacrificed and necropsied. Females that delivered a litter were necropsied on day 4 of lactation whilst all other animals were necropsied on presumed day 25 of gestation.
The necropsy included a gross examination of the external body surfaces, orifices and the cervical, thoracic and abdominal viscera.
The number of implantation sites was recorded. Uteri that appeared non-gravid were placed in 10% ammonium sulfide to reveal any implantation sites. If none were found the animal was considered to be non-pregnant.
Dead pups were removed, examined externally and discarded.

Statistical analysis

Body weight and food consumption data
A Bartlett's test was performed to determine if the dose groups had equal variance at the 1% level of significance. If the variances were equal, the testing was done using parametric methods, otherwise non-parametric methods were used.
The parametric procedure was a one-way analysis of variance. Dunnett's test was used to assess significance of differences between test and control. In addition, a standard regression analysis for linear response in the dose groups was performed. The regression also tested for linear lack of fit in the model.
For the non-parametric procedure a Kruskal-Wallis test was performed. If there were significant differences between the means a Dunn's Summed Rank test was used to determine which treatment groups differed significantly from control. In addition Jonckheere's test for monotonic trend in the dose response was performed.
Test for equal variance (Bartlett) was conducted at the 1% level of significance. All other tests were conducted at the 5% and 1% levels of significance.

Reproductive data and litter data
For number of implantation sites, gestation length, total number of pups per litter and number of live pups per litter, normal probability plots of the residuals by treatment group were used to judge whether or not departure from the assumptions of normality and homogenous variance were sufficient to invalidate the usual analysis of variance. If the usual analysis was invalid, a weighted General Linear Model (GLM) analysis was used, where the weight were proportional to the reciprocal of the variance. If the usual analysis was valid, the data were analyzed with a non-weighted GLM.

Result **:** There were no mortalities in the study and there were few treatment-related clinical signs of toxicity. These consisted of yellow, yellow/brown, yellow/orange or red/yellow stained coats for eight animals in the highest dose group. Alopecia was also noted in a few animals in this dose group.
Although there were no significant changes in growth rates in either the 50 or 150 mg/kg/day groups body weights were reduced in the highest dose group when compared to controls. The differences occurred from day 4 of gestation onwards and throughout lactation.
Body weight changes were also significantly less throughout gestation in the highest dose group when compared to controls. However, body weight changes for the highest dose group were significantly higher than controls through lactation. This was attributed to a cessation of exposure to the test material and recovery during this period.

There were no significant differences in absolute or relative food consumption for animals in the lowest dose group throughout gestation when compared to controls.

In the 150 mg/kg/day group absolute food consumption was unaffected but food consumption relative to body weight was significantly higher than that for controls during days 16-20 of gestation.

In the 500 mg/kg/day group Absolute food consumption was higher than controls during lactation. Relative food consumption was higher than controls from day 12 to the end of the study.

Skin irritation occurred in all dose groups during the study as follows:
50 mg/kg/day
Erythema slight to extreme (primarily slight to moderate)
Edema slight to moderate
Eschar slight to extreme (primarily slight to moderate)

150 mg/kg/day
Erythema slight to extreme (primarily slight to moderate)
Edema slight to extreme (primarily slight to moderate)
Eschar slight to extreme (primarily slight to moderate)

500 mg/kg/day
Erythema slight to extreme (primarily moderate to extreme)
Edema slight to extreme (primarily moderate to extreme)
Eschar slight to extreme (primarily moderate to extreme)
Fissuring in two animals for two days

Apart from skin irritation there were no other treatment-related findings at gross necropsy.

Reproduction and litter data are summarized in the following table.

	Dose group (mg/kg/day)			
Parameter	**0**	**50**	**150**	**500**
No. pregnant animals	15	11	10	12
No. that delivered	15	11	10	12
Mean gestation (days)	22.1	22.2	21.8	21.9
Mean No. implantation sites	16.0	15.6	17	16.6
No. litters with live pups	15	11	9	12
Mean No. live pups				
Day 0	14.9	13.5	14.9	15.1
Day 4 survival	97%	99%	99%	70%
Mean Wt. (g) live pups (adjusted)				
Day 0	6.55	6.65	6.10	5.56
Day 4	9.89	10.52	8.41	6.94
Proportion males (adjusted)				
Day 0	0.49	0.49	0.48	0.49
Day 4	0.49	0.48	0.47	0.51

In conclusion:
Developmental effects
At 150 mg/kg/day pup body weights were significantly lower than controls.
At 50 mg/kg/day there were no significant effects on litter data.

Maternal effects
At 50 mg/kg/day there was significant skin irritation in the parental animals.

The no observable adverse effect level for maternal toxicity was, therefore, less than 50 mg/kg/day and for developmental toxicity was 50 mg/kg/day.

Reliability **:** (2) valid with restrictions
The study was well reported but was only a screening study and did not cover fully the developmental toxicity endpoint.

(61)

Species	:	Rat
Sex	:	Female
Strain	:	Sprague-Dawley
Route of admin.	:	Dermal
Frequency of treatm.	:	Once daily
Duration of test	:	Days 0 through 20 of gestation
Doses	:	125, 250 & 1000 mg/kg/day
Control group	:	Yes
NOAEL maternal tox.	:	< 125 mg/kg bw
NOAEL teratogen.	:	250 mg/kg bw
Year	:	1994
GLP	:	Yes
Test substance	:	Sample F-220 consisted of: 77.7% saturates 22.3% aromatics

Method :

Undiluted test material was applied once daily to the clipped skin on the backs of groups of 15 presumed-pregnant female rats, aged 14-15 weeks, on days 0 to 20 of gestation at doses of 125, 250 mg/kg/day. A further group of 15 presumed-pregnant rats of the same age received test material at a dose level of 1000 mg/kg/day from day 5 through 9 of gestation. A group of 20 presumed-pregnant rats of the same age served as sham treated controls. Application sites were alternated (intrascapular and lumbar) to reduce skin irritation. Elizabethan collars were fitted to the rats for 6 hours after each application to reduce oral intake of test material. Any residual test material was wiped from the skin prior to collar removal.
Each animal was observed twice daily for signs of toxicity. Body weights were recorded on days 0, 4, 8, 12, 16 and 20 of gestation and food consumption was recorded for the periods 0-4, 4-8, 8-12, 12-16 and 16-20 days of gestation and days 0 and 4 of lactation.
Each litter was observed daily during days 0 through 4 of lactation for signs of toxicity and mortality.
Each female that was mated was sacrificed and necropsied. Females that delivered a litter were necropsied on day 4 of lactation whilst all other animals were necropsied on presumed day 25 of gestation.
The necropsy included a gross examination of the external body surfaces, orifices and the cervical, thoracic and abdominal viscera.
The number of implantation sites was recorded. Uteri that appeared non-gravid were placed in 10% ammonium sulfide to reveal any implantation sites. If none were found the animal was considered to be non-pregnant.
Dead pups were removed, examined externally and discarded.

Statistical analysis

<u>Body weight and food consumption data</u>
A Bartlett's test was performed to determine if the dose groups had equal variance at the 1% level of significance. If the variances were equal, the testing was done using parametric methods, otherwise non-parametric methods were used.
The parametric procedure was a one-way analysis of variance. Dunnett's test was used to assess significance of differences between test and control. In addition, a standard regression analysis for linear response in the dose groups was performed. The regression also tested for linear lack of fit in the model.
For the non-parametric procedure a Kruskal-Wallis test was performed. If there were significant differences between the means a Dunn's Summed Rank test was used to determine which treatment groups differed

significantly from control. In addition Jonckheere's test for monotonic trend in the dose response was performed.
Test for equal variance (Bartlett) was conducted at the 1% level of significance. All other tests were conducted at the 5% and 1% levels of significance.

Reproductive data and litter data
For number of implantation sites, gestation length, total number of pups per litter and number of live pups per litter, normal probability plots of the residuals by treatment group were used to judge whether or not departure from the assumptions of normality and homogenous variance were sufficient to invalidate the usual analysis of variance. If the usual analysis was invalid, a weighted General Linear Model (GLM) analysis was used, where the weight were proportional to the reciprocal of the variance. If the usual analysis was valid, the data were analyzed with a non-weighted GLM.

Result **:** There were no mortalities during the study.
The only clinical observation was dermal irritation which was observed in all dose groups as follows:

125 mg/kg/day	slight to extreme (primarily slight to moderate) erythema, edema, eschar and dry skin.
250 mg/kg/day	slight to extreme (primarily moderate to extreme) erythema, eschar and dry skin. Slight to moderate edema. Slight fissuring noted in one female on gestation days 5 through 10.
1000 mg/kg/day	Slight to extreme (primarily moderate to extreme) erythema, edema, eschar and dry skin.

There were no effects on either body weights or body weight changes in the 125 and 250 mg/kg/day groups. However body weight changes were significantly lower than controls for the 1000 mg/kg/day group during the period days 4 through 12 of gestation. From day 16 to day 20 of gestation, body weight changes were higher in the treated group than the controls.

Food consumption was unaffected in the 125 mg/kg/day group.
Minor differences in the 250 mg/kg/day group were not considered to be toxicologically significant.
In the 1000 mg/kg/day group absolute food consumption was lower than controls on gestation days 8 to 12 but was higher than controls days 16 to 20. This was accompanied by similar changes in relative food consumption. The higher values occurred after cessation of exposure to the test material.

The only finding at gross necropsy that was considered to be treatment-related was that of skin irritation.

The litter data are summarized in the following table.

	Dose group (mg/kg/day)			
Parameter	**0**	**125**	**250**	**1000**
No. pregnant animals	16	14	13	14
Mean gestation (days)	22.4	22.4	22.1	22.1
Mean No. implantation sites	16.1	16.8	17.2	17.7
No. litters with live pups				

	16	14	13	14
Mean No. live pups				
Day 0	14.4	14.9	16	15.8
Day 4 survival	93%	92%	93%	91%
Mean Wt. (g) live pups (adjusted)				
Day 0	6.33	6.45	6.36	6.33
Day 4	8.2	8.59	8.56	8.43
Proportion males (adjusted)				
Day 0	0.49	0.52	0.46	0.49
Day 4	0.51	0.51	0.49	0.50

Reliability : (2) valid with restrictions
The study was well reported but was only a screening study and did not cover fully the developmental toxicity endpoint.

(62)

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period : Days 0-19 of gestation
Frequency of treatm. : Daily
Duration of test : Days 0-19 of gestation
Doses : 15, 60, 250 & 500 mg/kg/day
Control group : Yes
Year : 1988
GLP : Yes
Test substance : Coker Light Gas Oil (CLGO) see section 1.1.1.

Method : Coker Light Gas Oil (CLGO) was applied daily to the shorn dorsal skin of groups of ten presumed-pregnant female rats (aged approximately 7 weeks) at the dose levels shown below and for the duration indicated. Since it was believed that inhalation of test material could be a confounding factor a second group of controls (remote controls) were housed in an area in which they could not inhale gas oil that had been applied to other animals.

Groups 1-6 inc. were designated prenatal toxicity groups and were sacrificed on day 20 of gestation. Groups 7 & 8 were postnatal toxicity groups and were sacrificed on day 4 post partum.
The bioavailability group animals were sacrificed on day 13 of gestation.
The study design is outlined as follows:

	Dose level (mg/kg/day)	**Dosing days***
Prenatal toxicity group		
Group 1	0 (Remote sham control)	0-19
Group 2	0 (Proximal sham control)	0-19
Group 3	15	0-19
Group 4	60	0-19
Group 5	250	0-15**
Group 6	500	10-12
Postnatal toxicity group		
Group 7	0 (Sham control)	0-19
Group 8	60	0-19
Bioavailability group (5 rats only)		
Group 9	500	10-12

* denotes days of gestation
** dosing was stopped after day 15 because of severe dermal irritation
Observations were made daily for clinical signs. Body weights and food consumption were recorded regularly throughout the study.

Each female in the prenatal toxicity groups were sacrificed on day 20 of presumed gestation and the thoracic and abdominal cavities were examined grossly.
The uterus and ovaries were removed and examined grossly. The number of corpora lutea per ovary for each rat was recorded. The ovaries of non-pregnant females were examined and then discarded. Uterus weights were also determined. The uterine contents of each pregnant rat were exposed and a record made of the number and location of all implantations.
At necropsy, blood samples were taken from all the animals and the following clinical chemical measurements were made:

Alanine aminotransferase	Glucose
Albumin	Iron
Albumin/globulin ratio	Lactate dehydrogenase
Alkaline phosphatase	Inorganic phosphorus
Aspartate aminotransferase	Potassium
Bilirubin (total)	Sodium
Calcium	Sorbitol dehydrogenase
Chloride	Total protein
Cholesterol	Triglycerides
Creatinine	Urea nitrogen
Globulin	Uric acid

Fetuses were examined and half were preserved for examination of soft tissue abnormalities, the remainder being differentially stained for skeletal examination.

Animals in the post natal groups (parents and offspring) were sacrificed on day 4 post partum. The thoracic and abdominal cavities of each dam were exposed and all organs were examined grossly for abnormalities. The uterus was excised and examined for total number of implantations. Thymus and liver weights were recorded. Although the pups were preserved in fixative, no evaluations were performed.

Animals in the bioavailability group were treated dermally with CLGO containing two radiolabelled markers on gestation days 10, 11 and 12 in metabolism cages. CLGO was applied at a dose of 500 mg/kg and each ml of CLGO contained 15.12 µCi of ^{3}H-BaP and 15.07 µCi of ^{14}C-carbazole.
24 hours after the third application of the labeled CLGO, the animals were killed and blood samples were collected. The amniotic fluid of each of the fetuses was collected. Embryo was separated from the yolk sac and placenta, embryos, amniotic fluid and yolk sacs were pooled for each dam and the weights and volumes were determined.
Maternal tissues collected for radioactivity analysis included: blood, thymus, liver, heart, brain, small intestine, large intestine, kidneys, spleen, stomach, ovaries, urinary bladder, lungs, muscle, retroperitoneal fat, femur and residual carcass. Urine, cage wash and fecal samples were collected from the metabolism cage and analyzed for radioactivity.

Statistical analysis
Maternal biophase data, cesarean section data and fetal data were evaluated statistically by analysis of variance followed by group comparisons using Fisher's exact or Dunnet's test.
Serum chemistry data were analyzed for analysis of variance followed by comparisons using Tukey's test.

For all statistical analyses, differences between control and treated groups were considered to be significant if the probability of the difference being due to chance was less than 5% ($p < 0.05$)

Result **:** The only clinical signs that were considered to be substance-related are those of skin irritation, which ranged from moderate to severe. Erythema, flaking, scabbing and thickening of the skin was observed in all groups exposed to CLGO. In those animals exposed to CLGO for more than 16 days fissuring, eschar and necrosis of the skin was also observed.
Growth rates were normal except for those of the 250 and 500 mg/kg day groups whose body weights were significantly less than controls at day 20 of gestation. The mean maternal weight gains throughout gestation are shown in the following table

	Dose group (mg/kg/day)					
	O(R)[1]	0(P)[2]	15	60	250	500
Day 0-20 weight gain of prenatal groups						
Mean	164	171	153	163	133[bd]	147[c]
SD	18	24	15	18	19	5
N [3]	10	9	10	9	9	10
Day 0-20 weight gain of post natal groups						
Mean		163		135[d]		
SD		14		24		
N		10		9		

[1] Remote control group
[2] Proximal control group
[3] No. of pregnant dams
[b] P <0.01 compared to group 1
[c] P <0.05 compared to group 2
[d] P <0.01 compared to group 2

The body weight changes of controls and treated groups in the post-partum animals were comparable.

Food consumption of the 500 mg/kg/day group was significantly less than controls throughout the study. Those animals in the 250 mg/kg/day group consumed less food than the remote control group during most of gestation up to day 13 after which they were consuming less food than either of the control groups.

At necropsy, thymus weights in the 250 mg/kg/day group were found to be less than the controls but the differences were not statistically significant. The authors did not consider the differences to be treatment related.

None of the following parameters for any CLGO dose group were different from control values:
- No. females aborted
- No. dams with viable fetuses
- No. dams with all resorptions
- Female mortality

No. corpora lutea
No. implantation sites
% preimplantation loss
No. viable fetuses
Litter sizes
Viable male fetuses
Viable female fetuses
Dead fetuses
No. resorptions
No. dams with resorptions

No effects were observed in any of the clinical chemical analyses.
Mean fetal body weights and crown-rump length measurements were unaffected by exposure of the dams to CLGO.
The anomalies observed in the fetuses were considered probably not exposure-related since they either occurred at a low incidence and/or also occurred in the control groups.

Reliability : (1) valid without restriction

(57)

5.9 SPECIFIC INVESTIGATIONS

Type	:	Initiation/promotion assay
Species	:	Mouse
Sex	:	Male
Strain	:	CD-1
Route of admin.	:	Dermal
No. of animals	:	30
Vehicle	:	Undiluted
Year	:	1993
GLP	:	Yes
Test substance	:	DGMK gas oil samples 1,6,7,9 & 11 (See section 1.1.1.)

Method : Five gas oil samples were investigated in this assay.
Groups of 30 male CD-1 mice were used for each treatment.
The following dosing regimes were used.

<u>Assessment of tumor initiating potential</u>
50 µl of each test material was applied undiluted to the shorn dorsal skin of the mice for 5 consecutive days of the first week of the initiating period.
A promoter [TPA (12-0-tetradecanoylphorbol-13-acetate)] was then applied twice a week (50 µg/animal, dissolved in 50 µl acetone) from week 4 to week 28.
As a positive control, DMBA was applied once (50 µg/animal, dissolved in 50 µl acetone) on the first day of the administration period and then followed by treatment with TPA as described above.

<u>Assessment of tumor promoting potential</u>
The animals were initiated by a single dose of DMBA (50 µg/animal dissolved in 50 µl acetone).
From week 4 to week 28, the test materials were applied undiluted twice a week at a dose of 5 µg/animal, dissolved in 50 µl acetone.
As a positive control TPA was applied twice a week instead of the test material.

Negative control animals were initiated with DMBA or acetone, followed by

promotion with acetone or TPA respectively.

During weeks 2 and 3 of the study, the animals were not treated allowing for regression of possible skin alterations that may have occurred during the initiating period.

Body weights were determined once weekly and each week a detailed examination of the skin was performed.
At the end of the study, the animals were assessed grossly, followed by histopathology of the skin and all macroscopic lesions.

Result : Survival of the animals was unaffected by exposure to the gas oil samples. Body weights were only transiently reduced in the positive controls and those treated with sample 7 (predominantly aromatic) and very slightly in animals treated with sample 9 (predominantly saturates).

During the initiation phase, the gas oil samples caused slight to moderate skin irritation consisting of reddening, scale formation, and/or erosions in all the gas oil groups except the group treated with sample 11 (predominantly saturates). Similar skin changes also occurred during the promotion phase.

During the two week recovery period between the initiation and promotion phase the skin changes in all groups were found to be reversible.

There were no treatment-related clinical findings other than skin changes.

The number of animals with neoplastic findings in the treated skin at the end of the study was follows:

Sample	**No. of animals with**			
	SCP*	**KAT****	**SCC*****	
Test for initiating activity				
Predominantly aromatics				
6	4	0	0	
7	14	0	0	
Predominantly saturates				
1	2	0	0	
9	5	0	0	
11	0	0	0	
Negative (Acetone/TPA)		0	0	0
Test for promoting activity				
Predominantly aromatics				
6	0	0	0	
7	7	2	0	
Predominantly saturates				
1	1	0	0	
9	0	0	1	
11	0	0	0	
Negative (DMBA/acetone)		0	0	0
Positive (DMBA/TPA)	30	0	0	

* SCP = Squamous cell papilloma
** KAT = Keratoacanthoma
*** SCC = Squamous cell carcinoma

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Appendix C.

Robust Summary

(Separate document)